FRONTISPIECE

Arcuate fertile hyphae of *Malbranchea aurantiaca* (UAMH 2844) x 1600.

FRONTISPIECE

Uncinate fertile hyphae of *Malbranchea aurantiaca*

(DAOM [illegible]) x 16[illegible].

THE UNIVERSITY OF ALBERTA

# TAXONOMY OF *MALBRANCHEA* AND SOME OTHER HYPHOMYCETES WITH ARTHROCONIDIA

by

© LYNNE SIGLER

A THESIS

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# ABSTRACT

The genus *Malbranchea* Saccardo is monographed and illustrated. The type species, *M. pulchella* is redefined and a new combination, *M. sulfurea* (Miehe) proposed for *M. pulchella* var *sulfurea*. Eleven new species are described and illustrated: *M. albolutea*, *M. arcuata*, *M. aurantiaca*, *M. chrysosporoidea*, *M. circinata*, *M. dendritica*, *M. flava*, *M. flavoroseus*, *M. flocciformis*, *M. fulva* and *M. gypsea*. A new heterothallic genus of the Gymnoascaceae is proposed for a single species, *Uncinocarpus reesii*, having a *Malbranchea* conidial state. The perfect states of some species of *Malbranchea* belong to the genera *Auxarthron* Orr, Kuehn and Plunkett and *Myxotrichum* Kunze of the Gymnoascaceae.

The relationship of *Malbranchea* to other genera is discussed and a tabular key to the genera provided. Two new genera are described. Included in the genus *Ovadendron* are the type species, *O. asperulatum*, as well as *Chrysosporium pannorum* (Link) Hughes and *Oospora sulfureo-ochracea* van Beyma. Two species are assigned to *Arcuadendron*, *A. ovatum*, the type species, and *A. triangularis*. The genus *Arthrographis* Cochet including the single species *A. langeroni* is reexamined and validated with a Latin description. *Oospora cuboidea* Sacc. et Ellis (= *Geotrichum microsporum* Smith) is transferred to this genus. Two species of *Myxotrichum* have *Oidiodendron* conidial states. The species, *M. setosum* (Eidam) Orr and Plunkett is rediscovered, and a new combination *M. striatosporus* (Barron

and Booth) is proposed. The genus *Scytalidium* is expanded to include *Geotrichum flavo-brunneun* Miller, Giddens et Foster and the *Scytalidium* state of *Hendersonula toruloidea* Nattrass (= *Exosporina fawcetti* Wilson), and a key to the species is given. The status and delimitation of a number of other genera is discussed: *Bahusakala*, *Briosia*, *Chrysosporium*, *Coccidioides*, *Coremiella*, *Geotrichum*, *Oidium*, *Oospora*, *Ptychogaster*, *Sporendonema*. The arthroconidial states of some Basidiomycotina are described.

# ACKNOWLEDGMENTS

Grateful appreciation is expressed to Dr. J.W. Carmichael whose expertise and knowledge of the taxonomy of Hyphomycetes has stimulated my own interest. His guidance and encouragement during the preparation of this thesis have been invaluable. Particularly helpful were his critical reviews of the manuscript and skilful clarification of some passages of the text.

My thanks are also extended to Dr. F.L. Jackson for providing the opportunity to undertake this project.

To other members of the Department of Medical Bacteriology, I express my gratitude for their willing assistance. In particular, Mrs. Suzanne Merlo skilfully translated some German text, and Dr. R.L.S. Whitehouse and Richard Sherburne offered helpful advice on some aspects of photography. Mrs. Debbie Berg expertly prepared Table I.

Thanks are also extended to other mycologists: Dr. A.S. Sekhon of the Provincial Laboratory of Public Health, who generously allowed me the use of his de Fonbrune micromanipulator, and Dr. G.F. Orr, Deseret Test Center, Dugway, Utah, who willingly provided many of the cultures used in this study. In addition, Dr. Orr was most helpful in locating some reference material.

Special appreciation is expressed to my husband, Doug, for his patience and encouragement during the past year.

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# INTRODUCTION

In 1882, Saccardo described the genus *Malbranchea* based on a fungus found growing on damp cardboard in France. In their brief description of the type species, *Malbranchea pulchella*, Saccardo and Penzig noted the arcuate nature of the fertile hyphae, "ramulis fertilibus in semicirculum curvis". However, they misinterpreted the arthric nature of the conidia and described them as "ex apice ramulorum continuo exsilientibus". They provided no illustrations. Although *M. pulchella* was described on two other occasions (Saccardo, 1908; Saccardo and Trotter, 1913), again without illustrations, it has been largely ignored by other mycologists.

A comprehensive and well illustrated account of a thermophilic *Malbranchea* by Cooney and Emerson (1964) again focused attention on this interesting genus. Cooney and Emerson precisely described the conidium development, "roughly every other one of these cells rounds up somewhat and develops an inner wall that approaches 0.6u in thickness but remains tightly united with the original, outer, hyphal wall". "A mature sporiferous coil, therefore, is composed of alternating thick-walled spores and thin-walled isthmuses. Our own observations show clearly that it is the final breaking of these thin-walled, sterile, connecting isthmuses that results in the separation and release of the spores."

In its formation of arthroconidia by conversion of existing hyphal elements, Malbranchea is related to a number of other genera of Hyphomycetes. The problem in distinguishing among these genera is one which afflicts the classification of Hyphomycetes in general. That is, the criteria for delimiting the genera are not clearly established. The class Hyphomycetes is part of the Deuteromycotina (Ainsworth, 1973), a group composed of the imperfect or conidial states of fungi, many of which are known to have perfect states in the Ascomycotina or Basidiomycotina. Genera of the Hyphomycetes are form genera, based on a morphological or anatomical stage in the life cycle of the fungus. Unfortunately the conidial form genera do not correlate exactly with the classification of the perfect states. Morphologically similar conidial states may have unrelated perfect states, and vice versa. Current classification of Hyphomycetes is ontogenetic, grouping together fungi having the same mode of conidium development. In such a scheme, emphasis is placed upon the nature of the conidiogenous cell from which the conidium develops.

A system of cross reference names is used when the connection between the conidial state and the perfect state is known (see Carmichael, 1962; Hennebert, 1971; Kendrick and Carmichael, 1973; Weresub, Malloch and Pirozynski, 1974; Nag Raj and Kendrick, in press). When the perfect state is known, the Linnaean binomial is applied to that state alone.

The imperfect or conidial state is cross referenced by referring to the (form-genus name) state of (Linnaean binomial).

The criteria for delimitation of the arthrosporic genera have not been precisely defined. Indeed, some genera are in need of major revision. For instance, the genus Geotrichum contains a large number of diverse fungi. Geotrichum has been revised to include only species having hyaline, slimy fission arthroconidia, consistent with the type species, G. candidum. Dematiaceous species are referred to Scytalidium, others elsewhere.

The presence of a supporting hypha or conidiophore distinguishes two genera, Oidiodendron and Arthrographis, and these genera differ from each other in their conidium development.

Malbranchea, in its formation of alternate arthroconidia, is closely related to Sporendonema and Coremiella. These genera are distinguished primarily on the basis of arthroconidium diameter and color. As defined here, Malbranchea species comprise a rather homogenous group having conidium diameters of mostly less than 4um.

Two new genera are proposed. Arcuadendron forms conidia in serial progression. The conidia may be either blastic or thallic. In the genus Ovadendron, conidia are formed in basipetal succession. The intercalary

arthroconidia are broader in diameter than the fertile hypha.

Taxonomic problems are encountered when species appear to intergrade between genera. These species are retained in the genus with which there is greatest overall similarity. Form genera are not biological entities but demes (Heslop-Harrison, 1962) defined by the taxonomist to separate fungi into groups for the purpose of indentification. If form genera are polythetic groups having in common a number of shared but not mutually exclusive characters, then intergrading species should be assigned to the form genus with which they have the greatest number of shared characters. For example, the *Oidiodendron* state of *Myxotrichum setosum* (q.v.) lacks the pigmented conidiophores of *Oidiodendron* but forms arthroconidia separated by the narrow connectives typical of *Oidiodendron*. This species is retained in *Oidiodendron* based on a number of other related characters. If a form genus were based on a single unique set of characters *i.e.* a monothetic group, then this species would be excluded since it differs in a definitive character. A new genus probably would be required to accommodate this species. Unfortunately, fungi do not conform to a rigid set of rules and exceptions to defined criteria are frequent. For this reason, form genera should be polythetic groups.

The same principle applies to the concept of the

species. In some species of *Malbranchea*, single strains differing in one or more characters from the group are retained within the species. For instance, four isolates of *Malbranchea aurantiaca* differ in some aspects of their colonial morphology but are indistinguishable in their microscopic appearance from others in the group. Until further isolates are examined there appears to be little justification for creating additional species for these strains.

Included within the genus *Malbranchea* are cellulolytic and keratinolytic species which are phylogenetically probably not closely related. Indeed, some cellulolytic species have their perfect states within the genus *Myxotrichum* of the Gymnoascaceae whereas some keratinolytic species have their perfect states in the genus *Auxarthron*.

## Terminology

The terms used in this report are primarily those recommended by the proceedings of the Kananaskis Conference on Fungi Imperfecti (Kendrick, 1971). However, aleurioconidium is retained here, in Vuillemin's (1911) original sense, for a conidium borne laterally or terminally on the undifferentiated hypha or on short pedicels and released by lysis of the supporting cell. Although aleurioconidium was rejected as a confused term, no other was proposed to replace it. The term refers to the method

of conidium dehiscence. (see Carmichael in Kendrick, 1971, p. 245).

Aleurioconidia are closely related to alternate arthroconidia and often intergrade. The term alternate arthroconidia is preferred to endoarthroconidia for arthroconidia separated by one or more relatively empty segments, and released by lysis of the outer wall of the intervening segment. Another suitable term is arthroaleurie defined by Orr (1963b).

Fission arthroconidia are formed by fission at a double septum of an existing hypha.

The term conidiophore is retained for a differentiated hyphal structure which supports the conidia or conidiogenous cells away from the vegetative mycelium (see Carmichael, in Kendrick, 1971, p. 47). The definition accepted by the Conference (see Kendrick, 1971, p. 227) was "a conidiophore is a system of conidiogenous cells, or a single conidiogenous cell, with or without differentiated supporting structures." A conidiophore without a differentiated supporting structure is called micronematous by Ellis (1971). A micronematous conidiophore cannot be distinguished from the vegetative hypha before the process of conidium formation begins. According to the definition used in this report, fungi developing conidia on undifferentiated hyphae lack conidiophores.

Following Hughes (1959), 1801 is taken as the starting point date for nomenclature of Hyphomycetes.

# MATERIALS AND METHODS

## A. Specimens and cultures

At the beginning of this study, the University of Alberta Mold Herbarium and Culture Collection contained approximately 100 strains of *Malbranchea* that were collected over a period of 10-12 years.

Many of them were isolated by Dr. G. F. Orr (Deseret Test Center, Dugway, Utah) during his continuing studies on Gymnoascaceae. During the study, he sent a further forty cultures.

Dr. Z. Hubalek, Institute for Parasitology, Prague, sent some of the isolates made during his extensive studies of birds (Hubalek, Balat, Touskova and Vik, 1973; Hubalek and Balat, 1974; Hubalek, 1974a,1974b). Some further isolates no longer available from Dr. Hubalek were received from Dr. Orr.

Mrs. C. A. Johansen of the Western Forest Products Laboratory Culture Collection of Wood Inhabiting Fungi kindly sent several cultures of *Ptychogaster*.

Dr. K. Tubaki of the Institute for Fermentation, Osaka, willingly exchanged a number of cultures.

Dr. C. T. Rogerson of the New York Botanical Garden Cryptogamic Herbarium lent the isotype specimen of *Oospora*

*cuboidea* Sacc. et Ellis. The Herbarium of the Commonwealth Mycological Institute, Kew, Surrey sent material and slides of *Oncocladium flavum* Wallr.

Further reference cultures were obtained from the Centraalbureau voor Schimmelcultures; the Commonwealth Mycological Institute; and the Department of Agriculture, Ottawa.

B. Abbreviations

ATCC -American Type Culture Collection, Rockville, Maryland, U.S.A.

CBS -Centraalbureau voor Schimmelcultures, Baarn, Netherlands

CDC -Center for Disease Control, Atlanta, Georgia, U.S.A.

CMI -Commonwealth Mycological Institute, Kew, Surrey, England

DAOM -Department of Agriculture, Ottawa, Ontario, Canada

DSA -Dextrose-salts agar

IFO -Institute for Fermentation, Osaka, Japan

NRRL -Northern Utilization Research Development Division, Peoria, Illinois, U.S.A.

NYBG -New York Botanical Garden Cryptogamic Herbarium, N.Y., U.S.A.

PYE -Phytone yeast extract agar

RSABG -Rancho Santa Ana Botanic Garden, Claremont,

California, U.S.A.

UAMH -University of Alberta Mold Herbarium and Culture Collection, Edmonton, Alberta, Canada

WFPL -Western Forest Products Laboratory Culture Collection of Wood Inhabiting Fungi, Vancouver, British Columbia, Canada

C. Disposition of type strains

Dried colonies of the type strain of each new species are maintained in the UAMH as holotype. Subcultures of the type strain are deposited in the CMI, CBS and ATCC.

D. Media

Media used in the study consisted of:

1. Phytone yeast extract agar, Baltimore Biological Laboratories (BBL) - dextrose 4%; phytone 1%; yeast extract 0.5%; agar 2%; and chloramphenicol (0.05 ug/l) and streptomycin (0.03 ug/l) (Carmichael, 1962).
2. Pablum mixed cereal agar - Pablum precooked cereal (Mead Johnson Nutritionals, Evansville, Illinois) 10%; agar 1.5%.
3. Dextrose-salts agar - 0.01% of each of dextrose, sodium chloride, ammonium sulfate and dibasic potassium phosphate in 2% agar solution, and biotin (0.1 ug/l). (Carmichael, 1962).

4. Oatmeal-salts agar - rolled oats 1%; magnesium sulfate 0.1%; potassium dihydrogen phosphate 0.15%; sodium nitrate 0.1%; agar 1.8%, pH 5.6 (Weitzman and Silva-Hutner, 1967; Padhye, Sekhon and Carmichael, 1973)

E. Standard culture conditions

Detailed descriptions of each strain were charted by recording the appearance under standard conditions.

1. Cellophane plates

A single sterile (62 x 62 mm) cellophane membrane designated 300 PT (plain, transparent) was layered over the surface of an agar plate prepared in a 90 mm plastic disposable petri dish (Carmichael, 1962).

The cellulolytic ability of the fungus was determined by its capacity to digest this membrane either partially or completely.

2. Inoculum

Two week old colonies on cereal slants prepared from frozen or lyophilized cultures were maintained at 8 C as stock cultures.

A scarcely visible inoculum was transferred from the periphery of the stock culture to the center of the cellophane membrane.

Each strain was inoculated in triplicate to PYE, cereal and oatmeal agar plates.

3. Temperature

A single PYE and cereal plate was incubated at each temperature of 25 C, 37 C and 45 C for a maximum of 21 days. A single oatmeal agar plate was incubated at 18 C, 25 C and 30 C for 35 days.

Thermophilic fungi can grow at a temperature of 50 C or higher, while only scant growth occurs at 20-25 C (Cooney and Emerson, 1964). Psychrophilic fungi are capable of growth at a minimum temperature of 0 C or lower and a maximum temperature of 20-25 C (Deverall, 1968).

4. Light

Cultures for standard descriptions and mating tests were exposed to fluorescent room light on an irregular basis, usually 8-10 hours per day, 5 days per week. Cultures at 18 C were incubated in darkness with exposure to light only during examination.

5. Incubation

Most cultures were incubated in the inverted position. The few that adhered poorly to the cellophane membrane were returned to an upright position.

Cultures on PYE and cereal were held for 21 days before being photographed and dried, and those on oatmeal agar for 35 days. If cleistothecial initials appeared or cleistothecia were not mature, oatmeal plates were kept 2-4 weeks longer.

Colonies were dried by removing the cellophane membrane to a press (Carmichael, 1963).

6. Standard descriptions

Each colony on PYE and cereal was examined weekly to measure the diameter and record colony characters and presence of a diffusable pigment.

The diameter, measured in two directions perpendicular to each other, was an average of the two readings.

7. Microscopic preparations

Permanent microscopic slides were prepared by the slide culture technique or by direct mounts from the colony in polyvinyl alcohol mounting medium (Carmichael, 1962).

Occasional non-permanent slides were prepared in lacto-

fuchsin (Carmichael, 1955) but the use of a phase contrast microscope alleviated the requirement for stained preparations.

8. Keratinolytic activity

The keratinolytic activity of a fungus *in vitro* was determined by the amount of digestion of the hair and the degree of penetration of the hyphae with or without the aid of penetrating bodies, when the fungus was grown on a nutritionally minimal medium (DSA) sprinkled with hair (Carmichael, 1962).

## F. Photography

All photomacrography was done with a single lens reflex camera mounted on an adjustable stand. Colonies were illuminated with a single 3200 K tungsten lamp at an oblique angle and photographed with Kodak Panatomic X, a fine grain moderate speed film. Illustrative plates were rephotographed using Panatomic X film and four 3200 K lamps.

Color transparencies were obtained using Kodak Hi Speed Ektachrome film, a reversal film for tungsten light. Some color resolution was sacrificed in conversion of transparencies to prints.

Kodak Hi Contrast Copy film, a fine grain high resolution film was used for all photomicrography.

Illustrative plates were rephotographed with Panatomic X film so as not to further increase contrast.

## G. Techniques for sexual crosses

Procedures for mating heterothallic species of the Gymnoascaceae especially of the genera *Arthroderma* and *Nannizzia* are well established (Dawson and Gentles, 1961; Padhye, 1969; Padhye and Carmichael, 1969, 1971, 1972, 1973). Early reports indicated that soil sprinkled with a keratin source such as feathers, hair, nail, hoof, *etc*. was superior to an agar medium (Dawson and Gentles, 1961; Dawson, Gentles and Brown, 1964; Stockdale, 1963). However, more recent studies (Weitzman, Kozma, and Silva-Hutner, 1969; Padhye *et*. *al*., 1973) indicate that a non-keratinous agar such as the oatmeal-salts agar (medium E) of Weitzman and Silva-Hutner (1967) stimulated excellent production of ascocarps.

The inoculum was from the periphery of a 14 day old culture grown at 25 C on cereal agar. Three different methods of inoculation to oatmeal-salts agar were attempted:

A. Premixed conidial suspensions were prepared by removing a small inoculum from each of two tester strains to 1 ml of sterile distilled water in a test tube. The suspension was mechanically agitated and pipetted onto the medium.

B. A conidial suspension of a single tester strain was prepared as in A, and a few drops from each of two strains pipetted to the agar plate, then mixed.

C. Loopsful from the conidial suspension of each of two tester strains were inoculated onto oatmeal agar in separate parallel streaks.

If the organism was known to utilize keratin some sterile hairs from an adult blonde woman were sprinkled over the agar.

Crosses were observed weekly to six weeks when cleistothecia were reported. Plates were retained up to 56 days before being discarded as negative.

## H. Single ascospore isolation

A single mature fertile cleistothecium removed to a welled slide containing 2-3 drops of sterile distilled water, was shaken to remove conidia and transferred to a second well where it was crushed. The suspension of ascospores and peridial hyphae was pipetted onto a PYE plate and single ascospores isolated with the aid of a de Fonbrune micromanipulator. After 24 hours incubation at 25 C, single germinating ascospores were transferred to PYE or oatmeal plates.

## I. Publication

This thesis is not intended to constitute valid publication of the new names and combinations proposed herein.

PART I THE GENERA

KEY TO THE GENERA .............................. Table I

## *Arcuadendron* Sigler et Carmichael gen. nov.

### Diagnosis

Vegetative hyphae hyaline or yellowish green, septate; conidiophores lacking. Conidiogenous cells are integrated, serial, indeterminate, progressive, growing from the apical conidium. Conidia are blastic, or thallic, serial, alternate, separated by short, branched or unbranched, hyphal segments and released by dissolution of the intervening cells. Conidia are broadly ellipsoidal or triangular with truncate ends, hyaline or yellowish in color, smooth or verrucose.

Type species: *Arcuadendron ovatum* Sigler et Carmichael sp. nov.

### Discussion

*Arcuadendron* differs from *Malbranchea* in that the fertile hypha increases in length after the formation of each conidium. In *Malbranchea* the hypha ceases growth before conversion to alternate arthroconidia occurs. In both, however, conidium dehiscence occurs by dissolution of

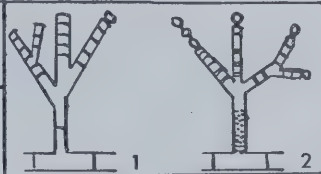
TABLE I

KEY TO THE GENERA

| Genera | Conidiogenous Hypha | Conidiogenous Cells | Conidia |
|---|---|---|---|
| 1 Arthrographis | Determinate | Retrogressive | Basipetal Chains |
| 2 Oidiodendron | Determinate | Retrogressive | Basipetal Chains |
| 3 Briosia | Determinate | Retrogressive | Basipetal Chains |
| 4 Scytalidium | Determinate | Retrogressive | Random or Simultaneous |
| 5 Bahusakala | Determinate | Retrogressive | Random or Simultaneous |
| 6 Geotrichum | Determinate | Retrogressive | Random or Simultaneous |
| 7 Basidiomycotina | Determinate | Retrogressive | Random or Alternate |
| 8 Coremiella | Determinate | Retrogressive | Alternate Chains |
| 9 Malbranchea | Determinate | Retrogressive | Alternate Chains |
| 10 Sporendonema | Determinate | Retrogressive | Alternate Chains |
| 11 Ovadendron | Determinate | Retrogressive | Alternate Chains |
| 12 Chrysosporium | Determinate | Retrogressive | Alternate or Single |
| 13 Arcuadendron | Indeterminate | Serial Progressive | Acropetal Chains |
| 14 Ptychogaster | Indeterminate | Serial Progressive | Acropetal Chains |

*On natural substrate but not necessarily in culture

TABLE I CONTINUED

<table>
<tr><th>Diameter of Conidium Compared to Fertile Hypha</th><th>Dehiscence</th><th>Color of Conidia</th><th>Differentiated Conidiophores</th><th>Figure</th></tr>
<tr><td rowspan="2">Same Width</td><td rowspan="6">Fission</td><td rowspan="2">Light Colored</td><td>Unpigmented</td><td rowspan="2"></td></tr>
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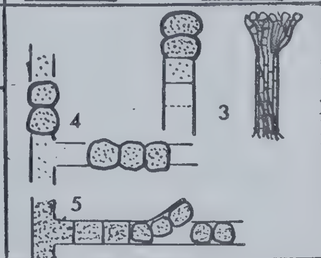
<tr><td>Broader</td><td rowspan="3">Dark Colored</td><td>Coremia*</td><td rowspan="3"></td></tr>
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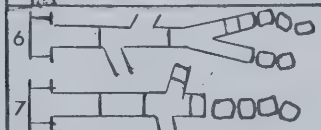
<tr><td rowspan="3">Hyaline</td><td rowspan="2"></td></tr>
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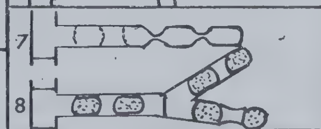
<tr><td rowspan="2"></td></tr>
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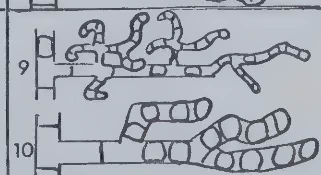
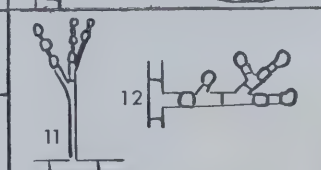
<tr><td rowspan="5">Light Colored</td><td>Absent</td><td></td></tr>
<tr><td rowspan="4">Broader</td><td>Unpigmented or Absent</td><td rowspan="2"></td></tr>
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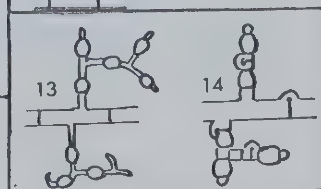
<tr><td rowspan="2"></td></tr>
<tr><td>Sporodochia*</td></tr>
</table>

the intervening segments.

Arcuadendron ovatum Sigler et Carmichael sp. nov.

Description

Colonies on PYE (Figs. 1A,1B) grow moderately rapidly (37-39 mm in 21 days) and are drab violet with white or pinkish violet center, reverse dark purple. In appearance, the colony is suede-like, flat with raised center and sharp outward radiating folds developing cracks near the center (Fig. 1A), or floccose, flat near the periphery, but rising to a central plateau, smooth (Fig. 1B). Growth at 37 C is similar, 25-36 mm in 21 days, drab violet, dark violet reverse, downy with few folds at the center or entirely cerebriform (Figs. 1C,1D).

On cereal, colonies (Figs. 1E,1F) attain the same diameter. Surface growth is violet, reverse dark violet, but the aerial growth is pale buff, floccose, slightly raised with flat central plateau and glabrous margin (Fig. 1E), or yellow, powdery and flat, sectoring near the margin (Fig. 1F). The violet surface pigment does not diffuse into the agar. Colonies at 37 C grow somewhat slower (18-32 mm at 21 days) and show less luxuriant aerial growth.

The optimum temperature range is 25 C - 30 C.

Vegetative hyphae are hyaline or greenish yellow, septate. Conidia are blastic, developing serially on undifferentiated hyphae (Fig. 1G). The fertile hypha is curved (Figs. 1H,1I), narrow, 1.0-1.5um in diameter, arising as a lateral branch from the broader vegetative hypha. Conidiogenous cells are undifferentiated from the fertile hypha, holoblastic. A marked increase in volume precedes delimitation of the conidium by a basal septum (Fig. 1J). The hypha continues to grow from the apical part of the conidium (Fig. 1J), often branching, and the conidium is delimited by an apical septum. Mature conidia (Fig. 1K), released by dissolution of the intervening hyphal segments are truncate, broadly ellipsoidal, verrucose at maturity, hyaline at first, later greenish-yellow, 3-4.5um x 4-7(9)um.

No other spore state was observed and an attempt to cross the isolates was unsuccessful.

Holotype: soil, India, UAMH 2737

## Habitat and Activities

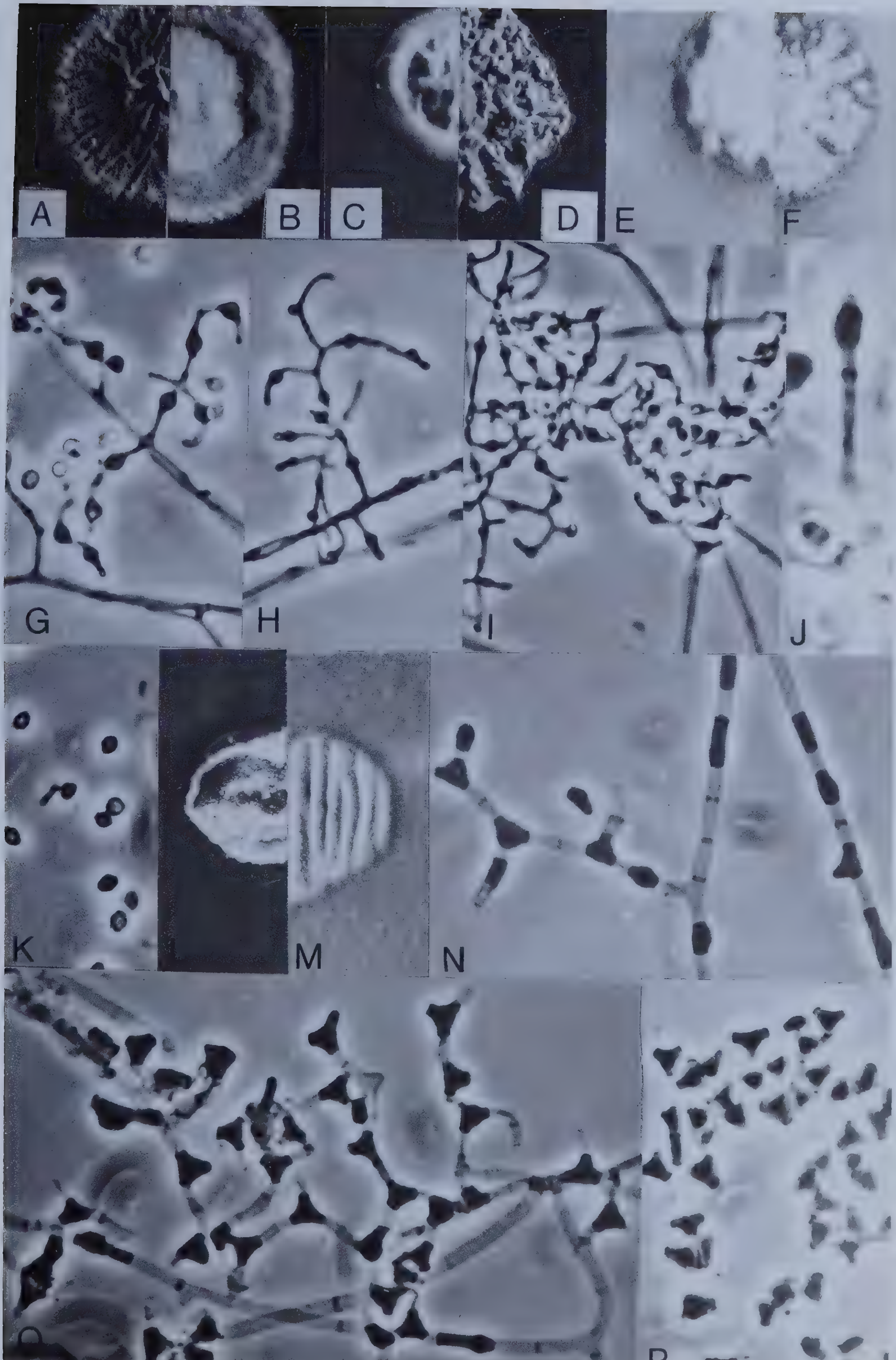
Two strains were isolated in India, one from soil, the other from an ulcer in a monkey.

*A. ovatum* is cellulolytic but does not attack keratin.

## Material Examined

UAMH 2737, soil, India, from H.C. Gugnani, National

Figure 1. A-K. _Arcuadendron ovatum_ (A,D,F,G,J-UAMH 2737; B,C,E,H,I,K-2746). L-P. _Arcuadendron triangularis_ (3433). Figs. 1A-1F. Colonial morphology after 21 days. A-D on PYE, E and F on cereal. C and D at 37 C, rest at 25 C. Figs. 1G-1I. Blastic conidia developing serially on curved fertile hyphae. Fig. 1J. Blastic conidium delimited by basal septum. Fig. 1K. Mature verrucose conidia. Figs. 1L-1M. Colonial morphology after 21 days at 25C. L on PYE, M on cereal. Figs. 1N-1O. Chains of triangular conidia developing serially on undifferentiated hyphae. Fig. 1P. Mature triangular conidia. Colonies x1.0, others x 830, except J, x 2300.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Institute of Communicable Diseases, New Delhi as S252; UAMH 2746, ulcer in a monkey, India, from H.C. Gugnani as M5259.

*Arcuadendron triangularis* Sigler et Carmichael sp. nov.

## Description

The colony (Fig. 1L) on PYE is slow growing, reaching a diameter of 20 mm in 21 days, creamy white, reverse light brown, raised and convoluted, dense, tough, powdery with tiny cracks across the surface. Pale yellow droplets of exudate appear on the surface.

On cereal (Fig. 1M) the colony is flatter, spreading to a diameter of 25 mm in 21 days with deep folds running parallel across the diameter of the colony, white, reverse buff, powdery.

There is no growth at 37 C; the optimum temperature is 25 C.

The vegetative hyphae are hyaline, septate, broad, 2.5-4um in diameter, often forming prominent racquet hyphae. Conidia are thallic, developing serially within an undifferentiated hypha, at first cylindrical in shape, then expanding in volume on one side to become triangular in shape (Fig. 1N). Meristematic growth continues until the conidium is delimited by a septum (Fig. 1,O). The curved

fertile hypha grows from the apex of the triangular conidium, forming conidia at regular intervals (Fig. 1,O). Conidia (Fig. 1P), released by disintegration of the intervening hyphal segments, are hyaline, smooth, triangular, more or less equilateral in shape, measuring 6-7um along the perpendicular from the apex to the base.

Holotype: hair of *Clethrionomys glareolus*, Hercegovina, Yugoslavia, Hubalek (BH68), 1970, UAMH 3433 (Orr-3351)

## Habitat and Activity

Only the single strain from Yugoslavia has been examined.

*A. triangularis* digests cellophane but fails to attack keratin.

## *Arthrographis* Cochet 1939

## Diagnosis

Vegetative hyphae septate, branched, hyaline. Conidiophores simple, hyaline, branched at the apex, appearing arborescent. Fission arthroconidia formed by disjunction and segmentation of hyaline fertile branches borne at the apex of the conidiophore, or formed by fragmentation of undifferentiated hyphae, are hyaline or

yellow, smooth, cylindrical, appearing discoid in end view.

Discussion

The genus *Arthrographis* proposed by Cochet (1939) for a single species, *A. langeroni* though adequately described and illustrated was invalidly published since Cochet did not include a Latin diagnosis. *Arthrographis* bears considerable resemblence to both *Geotrichum* and *Oidiodendron*. It differs from *Geotrichum* in forming dry fission arthroconidia on conidiophores. The conidiophores of *Arthrographis* lack the characteristic pigmentation of *Oidiodendron*. Most species of *Oidiodendron* develop rough-walled conidia some of which are strongly pigmented. Furthermore, *Arthrographis* lacks the connectives between maturing arthroconidia found in *Oidiodendron* (Barron, 1961, 1962; Cole and Kendrick, 1969).

Type Species

*Arthrographis langeroni* Cochet 1939, Ann. Parasit. Hum. Comp. 17:98-101 and pl. 3

= *Oidiodendron kalrai* Tewari et Macpherson 1971, Mycologia 63:602-611

Description

Colonies on PYE (Fig. 2A) are slow growing (22-28 mm at 21 days), yellow at first but characteristically buff or tan with tan reverse, slightly raised, dome shaped, downy or

tufted becoming velvety and tough, or powdery. The surface remains smooth or develops radial ridges or folds which may crack.

On cereal agar (Fig. 2B) colonies are flat, creamy white or buff, powdery, occasionally downy or woolly with tufts of upright hyphae, and reach a diameter of 36-44 mm in 21 days.

Growth at 37 C is slightly less rapid.

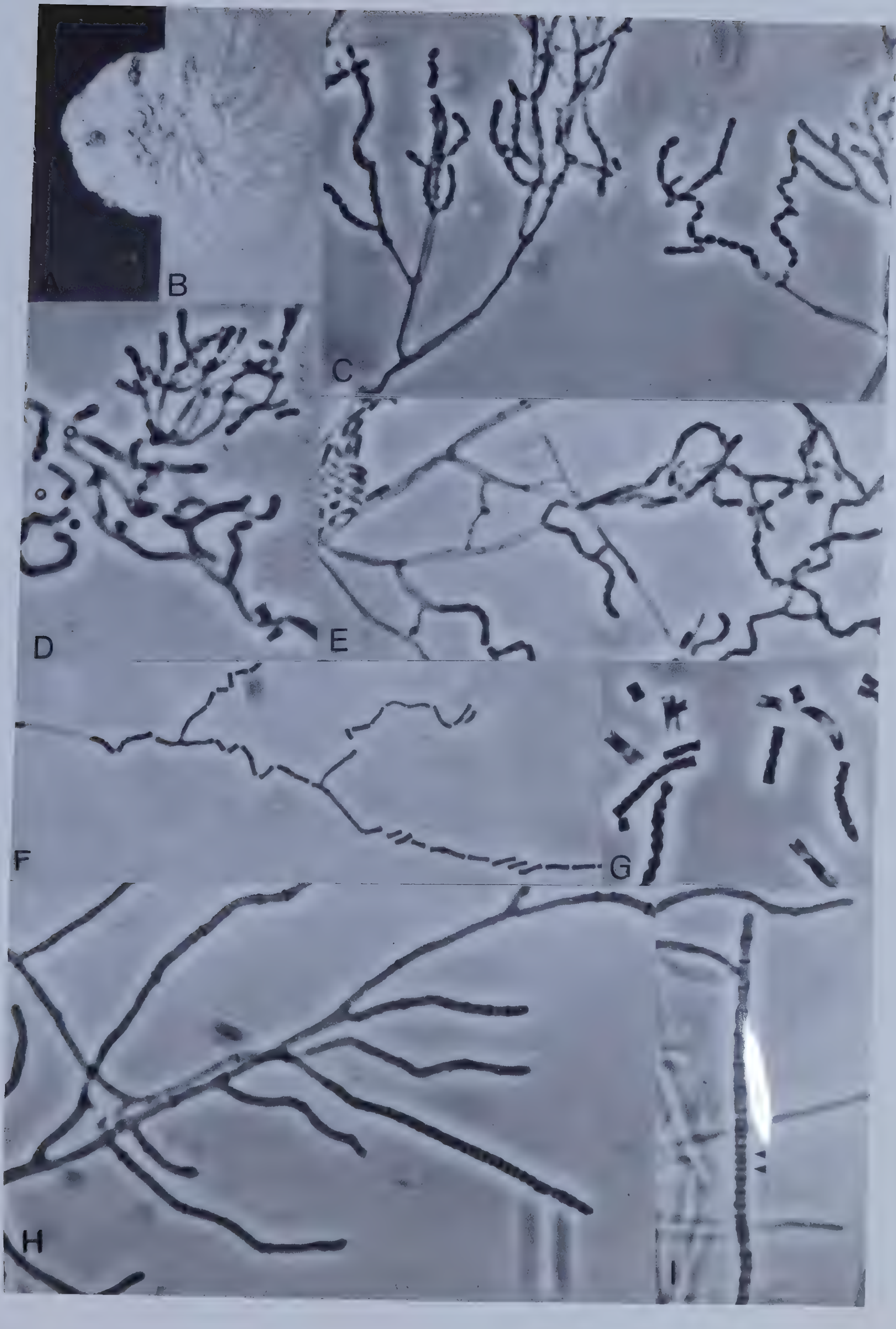
The vegetative hyphae are narrow, 1-3um in diameter. Arthroconidia, borne on short conidiophores developing multiple branches (Figs. 2C-2E), are cylindrical, hyaline, smooth, thin-walled, 1.5-2 x 2.5-4.5(5)um. During early growth, arthroconidia also develop by fragmentation of undifferentiated hyphae, but the conidia are longer and narrower (Fig. 2F). In degenerate strains, this form may predominate.

<u>Habitat and Activities</u>

Occurrence: <u>A. langeroni</u> has been recovered from soil, air, and human sources and appears to have a world-wide distribution.

Pathogenicity: The pathogenicity of this species has been studied by Cochet and by Tewari and Macpherson (1968b, 1971). Experimental inoculation into mice (Tewari and Macpherson, 1968b) resulted in severe neurological disorder,

Figure 2. A-F. Arthrographis langeroni (A,B-UAMH 3616; C-2617; D-3380; E-2610; F-2578). G-I. Arthrographis cuboidea (G-3760; H,I-676). Figs. 2A-2B. Colonial morphology after 21 days at 25 C. A on PYE, B on cereal. Figs. 2C-2E. Fission arthroconidia borne on branched conidiophores. Fig. 2F. Arthroconidia formed by fission of undifferentiated hyphae. Fig. 2G. Fertile hyphae and arthroconidia of isotype specimen of Oospora cuboidea. Fig. 2H. Septation of sporogenous hyphae at regular intervals. Fig. 2I. Subdivision of intervals by septa (arrows). Colonies x 1.0, others x 830.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more severe in mice inoculated intravenously than intraperitoneally. The organism was recovered from the spleen, kidney, liver, lung and brain. Tewari and Macpherson (1971) also discussed the dimorphic nature of the fungus. However, attempts to convert the type strain of _O. kalrai_ and Cochet's strain, a somewhat degenerate isolate, to the yeast phase by the _in vitro_ method outlined by them have been largely unsuccessful. Three morphological variants were isolated from sectors in Y-4, (ATCC 18434, UAMH 3616), the type of _O. kalrai_. All three formed mucoid colonies at 37 C, but only one (UAMH 3616c) formed few yeast-like budding cells after multiple transfers in brain heart infusion broth (Difco). The predominant growth throughout was hyphae and arthroconidia which often showed germination from the terminus.

Antigenicity: Tewari and Macpherson (1968a) observed significant protection in mice challenged with _Histoplasma caspulatum_ after vaccination with formalinized _O. kalrai_ vaccine (45% mortality) compared to 100% mortality with mice immunized by living _O. kalrai_. In contrast, the non-immunized controls had a mortality of 85%.

Further studies conducted by Tewari, Macpherson and Maitra (1969) demonstrated cross-reactivity of _O. kalrai_ with _H. capsulatum_. Guinea pigs sensitized by _H. capsulatum_ or _O. kalrai_ revealed cross-reactive hypersensitivity when the animals were skin tested by either histoplasmin or

oidiodendrin. In serological tests, antisera of _H. capsulatum_ reacted with both histoplasmin and oidiodendrin whereas antisera of _O. kalrai_ reacted only with oidiodendrin.

Activity: _A. langeroni_ does not attack cellophane, but digests keratin slightly often with penetration of the hair shaft by single hyphae.

Material Examined

From human sources: UAMH 3464, TYPE strain, chronic digital lesions, France, by Cochet, circa 1939, from CBS as strain Cochet 112.38; UAMH 3616, TYPE strain of _Oidiodendron kalrai_, from sputum of man with ill-defined respiratory ailment, India, Tewari (Y-4), from ATCC as 18434; UAMH 2298, also received as strain Y-4, listed as soil isolate, New Delhi, by Mohapatra, from C. Campbell, Harvard University; UAMH 2610, UAMH 2617, isolates from mixed culture also containing _Ctenomyces serratus_, received as Y-4, New Delhi, Mohapatra, from Tewari, Columbus, Ohio; UAMH 2546, gastric wash specimen, Edmonton, 1965; UAMH 3380, skin of right foot (isolated on 3 occasions), from L. Friedman, New Orleans as 192; UAMH 3715, human source, California, from Orr as O-2556;

From other sources: UAMH 3279, air sampling, Rabat, Morocco, by J. Chabert, from Nicot, Paris as 5216; UAMH 2578, from CBS as (CBS 344.49?) strain von Arx of _Ramularia gei_,

possible contaminant in culture

*Arthrographis cuboidea* (Sacc. et Ellis) Sigler comb. nov.

= *Oospora cuboidea* Saccardo et Ellis 1882, Michelia 2:576 (Basionym)

= *Geotrichum cuboideum* (Sacc. et Ellis) Sumstine 1913, Mycologia 5:56 and pl. 83

= *Coremiella cuboidea* (Sacc. et Ellis) Ciferri et Caretta 1960, Mycopath. et Mycol. Appl. 12(3):249 and Tab. 3

= *Geotrichum microsporum* Smith 1962, Trans. Brit. mycol. Soc. 45(3):388-389 and Tab. 8, fig. 4

= *Briosia microspora* (Smith) von Arx 1972, Ant. v. Leeuw. 38(3):293

non *Stysanus ulmariae* McWeeney 1895

= *Coremiella ulmariae* (McWeeney) Mason ap. Hughes

## History

In 1882 Saccardo and Ellis discovered *Oospora cuboidea* growing on rotting oak in Newfield, New Jersey and provided a brief description but no figures. In 1913, Sumstine examined the presumed isotype specimen and transferred the species to *Geotrichum* with a brief description and illustration.

In 1960, Caretta examined the isotype specimen of *O.*

cuboidea of Ellis (from the NYBG) and proposed a new combination Coremiella cuboidea (Sacc. et Ellis) Cif. et Caretta. Included in his concept of this species were four isolates from human feces, one of which (UAMH 829), received from Caretta in 1960 as Coremiella cuboidea, has been identified as a Trichosporon, possibly T. cutaneum. Indeed, Caretta's figures strongly suggest differences between the four human isolates and O. cuboidea. In placing Coremiella ulmariae in synonymy with O. cuboidea Caretta misinterpreted the manner of arthroconidium formation of the latter which he described as an endogenous process (see also Coremiella).

An examination of the isotype specimen of Oospora cuboidea (Fig. 2G) reveals this fungus to be conspecific with Geotrichum microsporum Smith. However, this species belongs neither in Geotrichum nor Coremiella; therefore, a new combination is proposed in Arthrographis.

Von Arx (1972) transferred G. microsporum to Briosia while simultaneously suggesting that Coremiella cuboidea (Sacc. et Ellis) Cif. et Car. and C. ulmariae (McWeeney) Mason apud Hughes could be conspecific with Briosia ampelophaga Cavara, the type of which he examined. However, Briosia differs from both in forming meristem arthroconidia (Kendrick, 1971, p. 168) in contrast to the fission arthroconidia of Arthrographis and the alternate arthroconidia of Coremiella (see also Briosia, Coremiella).

Description

Colonies on PYE grow rapidly, by 7 days reaching a diameter of 50-60 mm and becoming pale yellow, flocculent and powdery with tufts of sporogenous hyphae over the cellophane membrane. The reverse is yellow. Growth at 37 C is almost as rapid and similar in appearance except that the reverse becomes pink or violet by 14 days.

On cereal agar at 7 days, colonies are 60-70 mm, yellow, with dense, flocculent tufts of hyphae. The reverse at first is yellow. A pink pigment diffusing into the agar later stains the reverse of the colony dark blue. Growth is less rapid at 37 C (25-30 mm in 7 days).

Hyphae septate, hyaline, 2-5um in diameter. Conidiophores are scarcely differentiated, simple, hyaline, branching near the apex to form a tuft of sporogenous hyphae. The sporogenous hypha, 80-150um in length, delimited by a basal septum (Fig. 2H), is unbranched, at first sparingly, then more regularly septate (Fig. 2H), in more or less basipetal succession. The sporogenous hypha forms septae at regular intervals, each interval 3.5-5um wide, which further subdivide into uniform segments, 2-2.5um in width (Fig. 2I). The intervening septae, appearing at first less dense, begin to form before initial septation of the sporogenous hypha is complete (Fig. 3A), and develop randomly. Fission arthroconidia (Fig. 3B), formed by disjunction of the hypha in more or less basipetal

succession are square, or rectangular, often wider than long, discoid in end view, smooth, hyaline at first, later yellow, 1.5-2.5um x 2-3.5um.

Habitat and Activities

A. cuboidea has been reported from mine timber (Smith, 1962; Fassatiova, 1971) and from southern yellow pine timber (Chidester, 1940). It causes a pink stain in heartwood and sapwood of pine, birch, cyprus, hemlock, spruce, fir, oak and Douglas fir (Chidester, 1940).

A. cuboidea digests cellophane but does not attack keratin.

Material Examined

From wood: Oospora cuboidea, ISOTYPE, oak, Newfield, New Jersey, 1881, by Ellis (3536) from NYBG; UAMH 676, birch post, Chalk River, Ontario by K. Shields, 1956, from Hughes DAOM 64066; UAMH 3101, TYPE, Geotrichum microsporum, from rotton mine timber, Johannesburg, South Africa, 1955, received from CMI as 94091;

From mushroom bed: UAMH 3792, by K. Komatsu, 1964, from Tubaki, IFO, as 9190.

Bahusakala Subramanian 1958

Type Species

Bahusakala olivaceonigra (Berk. et Br.) Subramanian 1958, J. Indian Bot. Soc. 37:61-63

= Septonema olivaceo-nigrum Berk. et Br. 1873, J. Linn. Soc. 14:90

According to the description of Ellis (1971), Bahusakala is a dematiaceous Hyphomycete forming fission arthroconidia on simple, irregularly branched conidiophores. Fission arthroconidia are brown, 0, 1 or many septate, delicately furrowed or striate, sometimes rough walled, cylindrical or oblong, truncate or rounded at the corners. The type species, B. olivaceonigra occurs on members of the family Liliaceae, including Agave and Yucca.

Muller, Harr and Sulmont (1969) described another species, the Bahusakala state of Aulographina pinorum (Desm.) v. Arx et Muller which differed from the type species in having smooth arthroconidia and in growing on Pinus maritima. In addition, Muller et. al. (1969) described and illustrated (their Fig. 1) arthroconidium development occurring within the hyphal wall, that is, endogenously. They noted that the wall of the maturing conidium was difficult to differentiate from the outer wall of the original hypha. From their figures, mature conidia appear to be released from the terminal end of the enveloping hypha or by fracture of the hypha.

A single strain (UAMH 3812, CBS 499.66) isolated from

*Mangifera indica* was received from the CBS as *Bahusakala olivaceonigra*. However, this isolate more closely resembles the *Bahusakala* state of *Aulographina pinorum* rather than *B. olivaceonigra* as described by Ellis (1971).

Arthroconidia of this strain develop on more or less undifferentiated hyphae either terminally or in an intercalary position. Hyphae are pale brown at first, becoming thick walled, yellow brown in color in the fertile segments. The hyphae are broad and sparingly septate, and cytoplasmic contents of some segments appear less dense.

Muller *et*. *al*. (1969) suggested that formation of arthroconidia in *A*. *pinorum* was endogenous. A similar mode of development was observed in UAMH 3812. The fertile segments of the hyphae, at first sparingly septate, cleave into smaller segments (Fig. 3C) and mature arthroconidia are released by dissolution or fracture of the outer wall (Figs. 3E,3F). Remnants of the outer wall are often visible around the released conidia, and conidia occasionally remain connected by the outer wall (Fig. 3D) in groups of 2's or 3's. In addition, arthroconidia form directly by the segmentation and disjunction of segments of the fertile hypha (Fig. 3G). There appears to be no outer wall surrounding these arthroconidia.

Rarely, within a broad hypha in which the cytoplasmic contents are less granular, an intra-hyphal hypha (Buller, 1933) breaking into arthroconidia (Fig. 3H) can be observed.

Figure 3. A-B. _Arthrographis cuboidea_ (A-UAMH 3101; B-676). C-I. _Bahusakala olivaceo-nigra_ (3812). Fig. 3A. Random development of intervening septa. Fig. 3B. Mature fission arthroconidia. Fig. 3C. Septation of undifferentiated fertile hypha. Fig. 3D. Mature conidia, remaining connected in groups of 2's or 3's by sections of the outer hyphal wall. Figs. 3E-3F. Mature arthroconidia released by dissolution or fracture of the outer hyphal wall. Fig. 3G. Fission arthroconidia formed by segmentation of undifferentiated hypha. Fig. 3H. Intra-hyphal hypha fragmenting to form arthroconidia. Fig. 3I. Fragmentation of new hyphal branch after fracture of the outer hyphal wall. All x 830, except H, x 2300.

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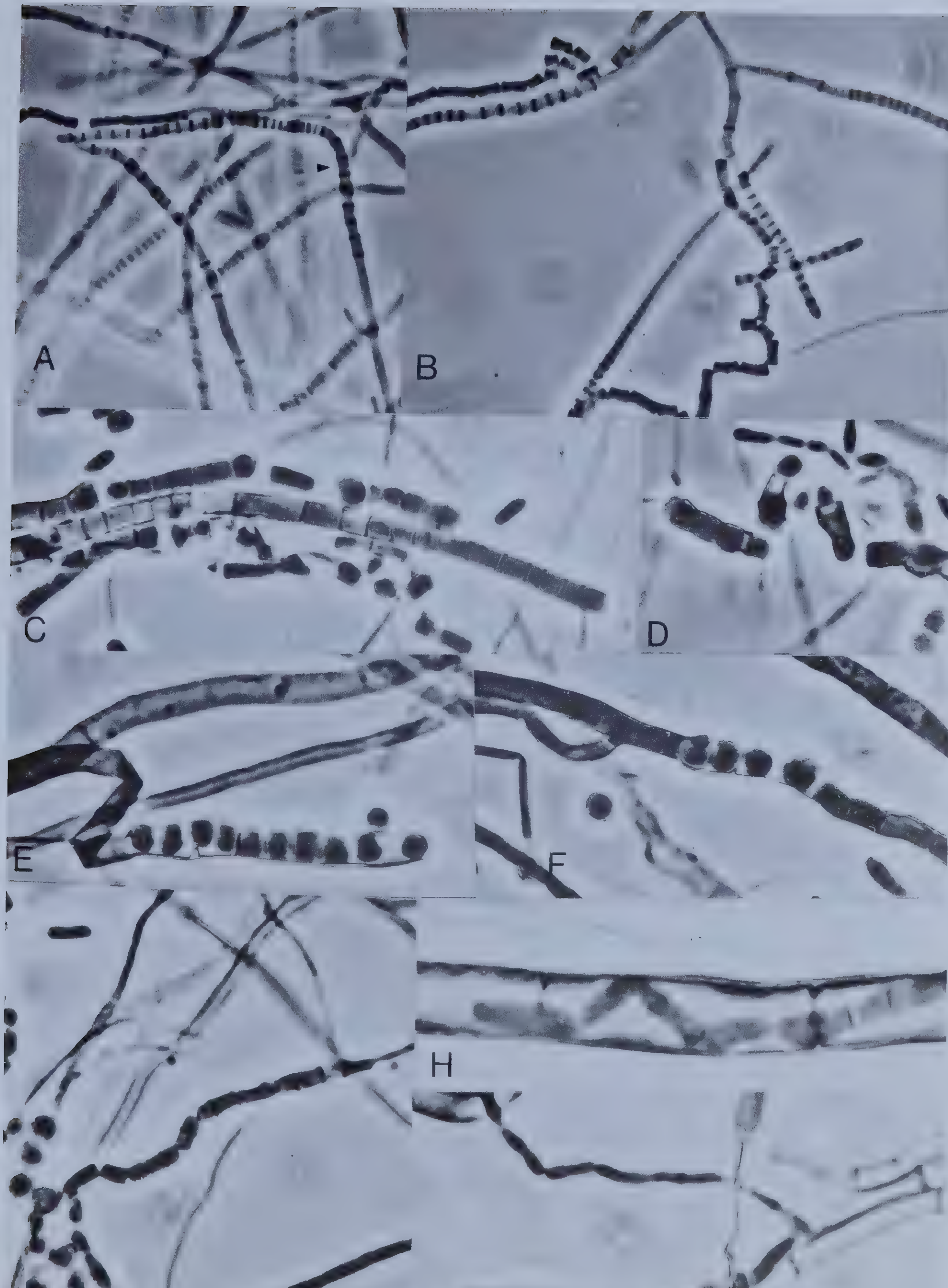
[illegible] arthroconidia. Fig. 3C. [illegible] of undifferentiated [illegible] hypha. Fig. 3D. Mature co[illegible] [illegible] the outer hyphal wall. Figs. 3E-3F. Mature art[illegible] [illegible] by dissolution or fracture of the outer hyphal wall. [illegible] undifferentiated hypha. Fig. 3H. [illegible] arthroconidia. Fig. [illegible] outer hyphal wall. All x [illegible] except [illegible], x 2300.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A similar situation was recorded by Lowry and Sussman (1966) in a mutant of *Neurospora crassa*. They observed that the hyphae invaded by intra-hyphal hyphae had sparse cytoplasmic contents and concluded that the invaded hyphae were moribund. They also reported that intra-hyphal hyphae were capable of penetrating the septal plugs of the invaded hypha as well as the outer wall. The frequency of intra-hyphal hyphae in the mutant of *N. crassa* was attributed to the periodic growth pattern of the isolate.

A similar phenomenon is evident in UAMH 3812. Many hyphae appear moribund, at least in segments. It is possible that the endogenous arthroconidia are actually formed by fission of the intra-hyphal hyphae growing within segments of the original outer wall. As arthroconidia are released from the outer wall, new branches also emerge (Fig. 3I) and this new growth is probably an extension of the intra-hyphal hypha. The extruded endohypha also divides to form arthroconidia (Fig. 3I).

Mature arthroconidia are pale brown, smooth, cylindrical with rounded corners, 0-1 septate, 3-4.5um x 5-10um. No other spore state was observed.

## Arthroconidial states of Basidiomycotina

## History

In 1889, Brefeld illustrated the conidial states of a number of Hymenomycetes, in which the hyphae divided into short segments called oidia. Hughes (1953) in his experimental classification of the Hyphomycetes discussed the formation of oidia by Basidiomycotina in his Section VII. This group comprised fungi in which the development of conidia occurred by septation and disjunction of simple or branched hyphae.

Brodie (1936) studied oidial development in *Collybia velutipes* and some other Hymenomycetes. He found that the oidia of *Collybia velutipes* occurred on both dikaryon and monokaryon mycelia but that clamp connections rarely occurred on the fertile branches of the dikaryon mycelium. He concluded that the oidia of the dikaryon mycelium were haploid.

Nobles (1948) studying the conidial states of a number of wood rotting fungi growing in agar culture, described the oidia of 13 species.

Maxwell (1954) in a study of Thelephoraceae observed that oidia were the most common form of asexual reproduction in the Agaricaceae and Polyporaceae of the Hymenomycetes.

In the UAMH, a number of strains have been accumulated as '*Geotrichum* sp.' which appear to be arthroconidial states of Basidiomycotina. Considerable problems arise in trying

to place these fungi in form-genera already described. When the arthroconidial states of Basidiomycotina have been more thoroughly studied, some additional genera may be required to accommodate these fungi.

Because the colonial morphology of the arthroconidial states is rather uniform, only one description is provided even though the microscopic morphology is variable. Although a number of isolates have been examined, no attempt was made to include all the known arthroconidial states of Basidiomycotina.

## Description

Colonies on PYE (Figs. 4A,4B) grow rapidly (35-62 mm in 7 days and almost filling the petri dish by 14 days) and are white or pale tan, reverse white or yellow. Aerial growth is dry, dense, patchy, matted, woolly or downy, raised, and often more dense on the cellophane than on the exposed medium. When dried, the aerial growth flattens becoming felt-like in texture, turning mottled yellow or tan in color. Most strains produce a diffusing yellow pigment and occasionally copious yellow exudate on the surface. A distinct sweet odor is apparent.

Growth on cereal is slightly more rapid (60-74 mm or filling the petri dish by 7 days) but otherwise similar. The diffusing pigment turns the medium tan in color.

Most strains are incapable of growth at 37 C, but some show slight growth. Most are strongly cellulolytic, markedly weakening the cellophane by 7 days.

Isolates which appear to be Basidiomycotina monokaryons are distinguished by the rapid growth and woolly or felt-like texture of the colonies, the strong odor and cellulolytic activity. On the basis of colonial morphology, the isolates comprise a rather uniform group. However, several variations in arthroconidium development were observed in the strains examined.

a) Group 1 (Strains 448, 507, 511, 1535, 1537, 2098, 2536, 2705, 2769, 2871, 3251, 3436, 3657)

Microscopically, these strains resemble _Geotrichum_ in their arthroconidium development. Fission arthroconidia (Fig. 4D) are formed by septation and disjunction of undifferentiated fertile lateral branches. Arthroconidia are hyaline, barrel shaped or cylindrical, single-celled, measuring 2-4(5.5)um x (2)3-10(15)um. Arthroconidia tend to lie in zig-zag (Fig. 4F) chains, and collapse easily when exposed to air. Thick walled intercalary or terminal chlamydospores (Fig. 4C) are produced by many strains. A single isolate (UAMH 2769) formed clamp connections.

These isolates are not included in _Geotrichum_ since the conidia are dry (see _Geotrichum_).

b) Group 2 (Strains 3776, 3787)

This group is the conidial state of Phlebia radiata Fr.

Colonies of these strains grow less vigorously, developing less luxurient aerial growth.

The conidial state of Phlebia radiata microscopically resembles Group 1. Arthroconidia are borne in chains on undifferentiated lateral branches which are often curved (Fig. 4E). Fission arthroconidia are hyaline, cylindrical, 2.5-4um x 4-9um. Clamp connections occur in both strains.

c) Group 3 (Strains 645, 2772, 3412)

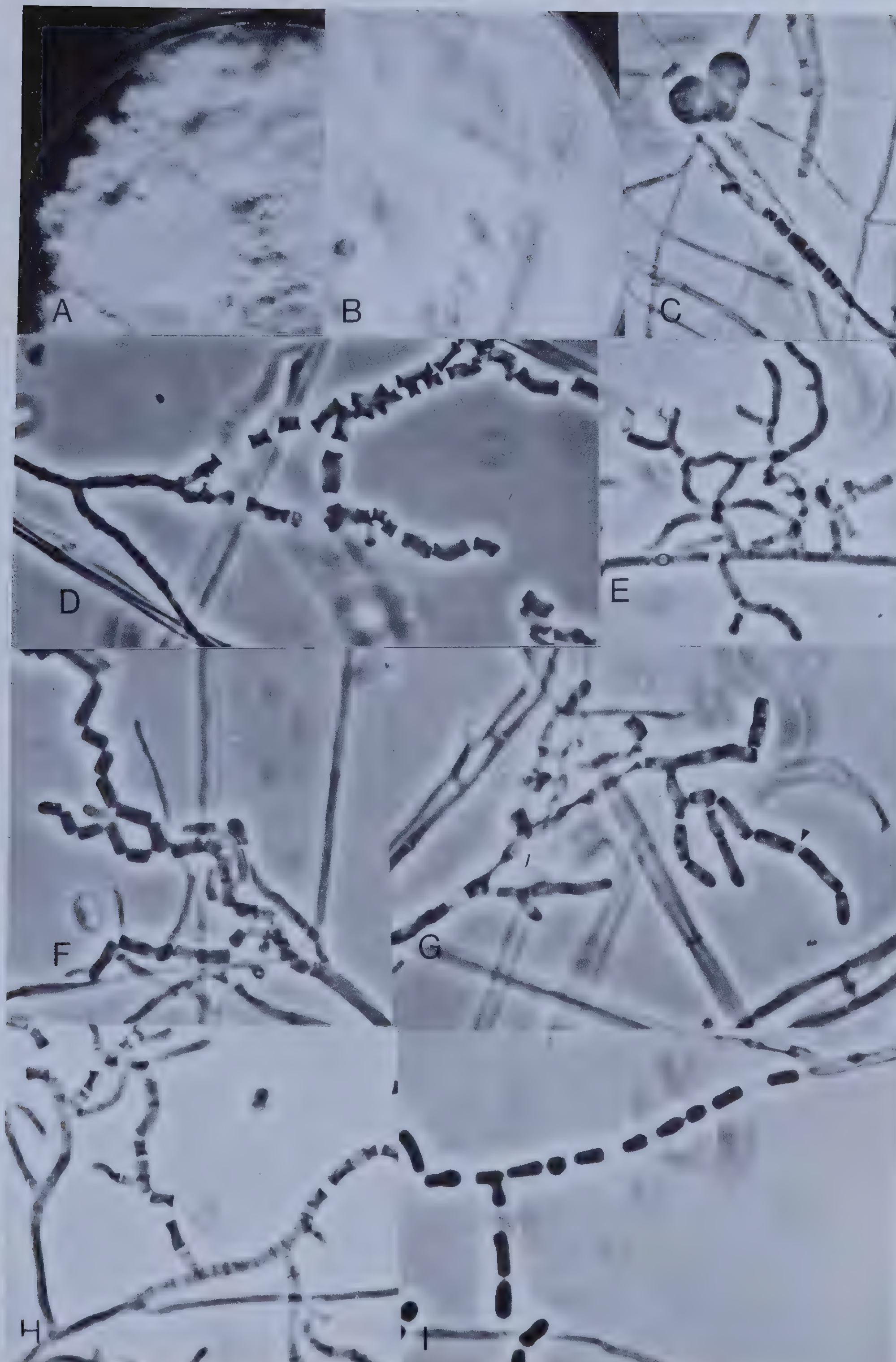
Arthroconidium formation in this group differs from groups 1 and 2 in the development of narrow separating cells between maturing arthroconidia (Fig. 4G). The disjunctor cells develop after septation of the fertile hypha (Fig. 4H). Arthroconidium development in this group appears similar to Oidiodendron. The connecting cell is often difficult to detect suggesting that it could be a gelatinous secretion of the maturing arthroconidia similar to the type described for Oidiodendron (see also Oidiodendron).

d) Group 4 (Strains 514, 3796, 3837)

This group contains two strains of Collybia conigena (Pers. ex Fr.) Bres., one of which (UAMH 3837) failed to sporulate.

Figure 4. Arthroconidial states of Basidomycotina. A-B. Colonial morphology (A-UAMH 3251; B-511). C,D,F. Group 1 (C-1537; D-2536; F-1535). E. Group 2 (3776). G-H. Group 3 (G-3412; H-645). I. Group 4 (3796). Figs. 4A-4B. Colonial morphology on PYE after 21 days at 25 C. Fig. 4C. Thick walled chlamydospores. Fig. 4D. Fission arthroconidia borne on undifferentiated hyphae. Fig. 4E. Fission arthroconidia borne on curved lateral branches. Fig. 4F. Mature fission arthroconidia in zig-zag chains. Figs. 4G-4H. Disjunctor cells developing between arthroconidia. Fig. 4I. Mature arthroconidia separated by collapsed disjunctor cells. Colonies x 1.0, others x 830.

[illegible] of Basidomycotin[illegible] [illegible] B-511). C,D,F. [illegible] F. Group 2 (3776). G-H. [illegible] Group 4 (3796). Figs. 4A-[illegible] after 21 days at 25 C. [illegible] [illegible]res. Fig. 4D. [illegible] [illegible] undifferentiated hyp[illegible] [illegible] borne on curved lateral b[illegible] Mature [illegible]ion arth[illegible] [illegible] cells developing bet[illegible] Fig. 4I. Mature arthrocon[illegible] [illegible] cells. [illegible]olonies x 1.0, [illegible]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The oidium development described by Brodie (1936) for *Collybia velutipes* (Curt. ex Fr.) Quel. is identical with this group. Following cessation of growth of the fertile hypha, the protoplasm of the hypha becomes condensed in segments separated by vacuoles which widen to form a separating cell. Brodie (1936) reported that transverse septa were rarely formed in the fertile hypha and speculated that release of the mature conidium was by dissolution of the outer wall of the separating cell.

The terminal walls of the arthroconidium are apparently formed *de novo*. Although in some respects resembling *Oidiodendron*, arthroconidium development in *Oidiodendron* is preceded by septation of the fertile hypha. The mature arthroconidia of group 4 (Fig. 4I) resemble the alternate arthroconidia of *Sporendonema* but the arthroconidia of this species are also formed by septation of the fertile hypha. Conidium dehiscence appears to be the same however.

Mature conidia (Fig. 4I) are variable in shape, cylindrical or rounded at the corners, or dumbbell shaped, 2.5-4um x 5.5-13um.

## Habitat

Isolated as probable contaminants in clinical material from human and animal sources, and from soil of sheep and horse corrals, and feathers of sparrow, in Alberta, Kentucky, South Carolina, Ohio, Kansas, Georgia, California

and Czechoslovakia.

### Material Examined

Material of *Collybia conigena*: UAMH 3796 (CBS 146.29) and UAMH 3837 (CBS 108.13); Material of *Phlebia radiata*: UAMH 3776 (CBS 278.29) and UAMH 3787 (CBS 285.73);
Alberta isolates from clinical specimens: UAMH 448, skin from perianal region, 1955; UAMH 507, sputum, 1956; UAMH 511, toenail, 1956; UAMH 514, blood, 1956; UAMH 645, toes, 1959; UAMH 2536, skin scrapings, canine, 1965; UAMH 3251, urine, 1969; From clinical specimens: UAMH 1535, sputum, from Orr as KCFS 62-5241; UAMH 1537, sputum, from Orr as KCFS 62-5706; UAMH 2705, vitreous humor of eye, Ohio, from J. Schwarz, Univ. of Cincinnati, Ohio; UAMH 3657, Orr-1209; From other substrates: UAMH 2098, California by P. Martin, 1961, as 840; UAMH 2769, sheep pen, Alberta by D. Remington, 1967; UAMH 2772, horse corral, Alberta by D. Remington, 1967; UAMH 2871, soil of broomsedge field, S. Carolina, 1967, from D. Coleman, Savannah River Ecology Laboratory, S. Carolina as G80209; UAMH 3436, feather of house sparrow, Valtice, Czechoslovakia, 1970, by Hubalek, from Orr as 0-3279

## *Briosia* Cavara 1888

### Type species

<u>Briosia ampelophaga</u> Cavara 1888, Atti Ist. Bot. Univ. Pavia, ser 2, 1:321-322, Tab. 5; Lindau 1910, in Rabenhorst's Kryptogamen-Flora 1(9):372; Sutton 1973, Mycol. Papers CMI 132:84-85, fig. 40.

non <u>Briosia cubispora</u> (Berk. et Curt.) von Arx 1972

≡ <u>Cladosporium cubisporum</u> Berk. et Curt. ap. Berk. 1875

≡ <u>Coremiella cubispora</u> (Berk. et Curt.) Ellis 1971

In the development of the arthroconidia of <u>Briosia</u>, meristem arthroconidia of Kendrick (1971, p. 168), septation occurs successively from the apex of the hypha followed by enlargement or expansion <u>i.e</u>. meristematic growth of the apical and subsequent segments. Expansion occurs only after delimitation by a septum. Mature arthroconidia are brown, thick-walled, globose or sub-globose or cylindrical and disarticulate by fission, at maturity, 5-7.5um x 6-11um (5-8um in diameter, see Sutton, 1973).

Morris (1963) illustrated meristematic growth occurring before delimitation by a septum in the manner described for <u>Basipetospora rubra</u> by Cole and Kendrick (1969) and Kendrick (1971, p. 168).

Von Arx (1972) placed <u>Coremiella</u> in synonymy with <u>Briosia</u> but <u>Coremiella</u> forms alternate arthroconidia rather than meristem arthroconidia. (See also <u>Arthrographis</u>.)

Sutton (1973) noted the similarity of conidium

development in *Briosia* and *Ojibwaya* Sutton. The latter differed in forming conidiophores from the basal cells of the erect funnel-like wall of the immersed stroma. The conidiophores were not borne on a synnema as in *Briosia*. Sutton (1973) considered *Briosia ampelophaga* to be closely related to *Coremium luteolum* Camara apud Serafim collected from *Vitis* in Portugal. However the lack of type material hindered positive identification.

## Habitat

*Briosia ampelophaga* occurs primarily on leaves of *Vitis* and *Platonia* causing leaf spot, and sometimes on fruits of *Vitis viniferae*. It has been collected in Brazil, Italy, Wisconsin and Illinois.

## Material Examined

UAMH 3822, on leaves of *Vitis riparia*, Daneville, Illinois, from DAOM as 137503, collected by Solheim.

## Other Species

*Briosia azaleae* (Peck) Dearness 1941, Mycologia 33:360

= *Periconia azaleae* Peck 1833, Ann. Rep. N.Y. State Mus. 25:93

= *Sporocybe azaleae* (Peck) Saccardo 1886, Syll. Fung. 4:608

Though I have examined no material, Dearness in his description noted few differences from _B_. _ampelophaga_. The arthroconidia of _B_. _azaleae_ however, are smaller, measuring 3-7.5um x 2.75-4.5um. Furthermore, _B_. _ampelophaga_ occurs commonly on grape leaves whereas _B_. _azaleae_ has been recovered from bud and twig blight of azalea (Davis, 1939).

Excluded Species

_Briosia_ _cystopoides_ (Bubak et Krieger) v. Arx 1972

= _Coremiella_ _cubispora_ q.v.

_Briosia_ _microspora_ (Smith) v. Arx 1972

= _Arthrographis_ _cuboidea_ q.v.

## _Chrysosporium_ Corda 1833

Type Species

_Chrysosporium_ _merdarium_ (Link) Carmichael 1962, Can. J. Bot. 40:1160 and figs. 30-34

= _Sporotrichum_ _merdarium_ Link 1818, Jahrb. Gewachsk. 1:176

= _Chrysosporium_ _corii_ Corda 1833, Sturm's Deutschl. Flora III (Pilze), Bd. 3, Heft. 13:85

For further synonymy and descriptions of other species of _Chrysosporium_, refer to Carmichael (1962). _Chrysosporium_ differs from _Malbranchea_ in forming aleurioconidia borne

terminally or on short or long lateral branches or directly on the sides of the hyphae. The aleurioconidia of *Chrysosporium* are broader than the diameter of the fertile hypha. Aleurioconidia intergrade with alternate arthroconidia which may predominate in some species.

*Other Species*

*Chrysosporium pannorum* (Link) Hughes 1958, Can. J. Bot. 36:749 = *Ovadendron pannorum* q.v.

## *Coccidioides* Stiles 1896

*Type Species*

*Coccidioides immitis* Stiles in Rixford and Gilchrist 1896, Johns Hopkins Hospital Report 1:209-269

*C. immitis* is a human pathogen that occurs in tissue in the form of globose cells which divide internally to produce a few to many endospores. These structures were at one time interpreted as asci and ascospores (see Dodge, 1935, p. 147) but no one has demonstrated karyogamy or meiosis in connection with them. Currently, the endosporulating cells are referred to as 'spherules'.

It seems likely that the spherules represent an unusual adaptation to parasitic growth, with multiplication by endosporulation instead of budding (as in most fungal

pathogens) or fission arthroconidia (as in the dermatophytes). Other characters of *C. immitis* suggest that it may be a heterothallic member of the Gymnoascaceae, whose sexual state has not yet been observed.

In culture, *C. immitis* grows as a mold and produces arthroconidia typical of the form-genus *Malbranchea*. A description of the conidial state and further discussion are given under the *Malbranchea* state of *Coccidioides immitis*.

## *Coremiella* Bubak et Krieger 1912

Type Species

*Coremiella cubispora* (Berk. et Curt.) Ellis 1971, in Dematiaceous Hyphomycetes p. 33, fig. 6

≡ *Cladosporium cubisporum* Berk. et Curt. apud Berk. 1875, in Grevillea 3:107 (Basionym)

= *Coremiella cystopoides* Bubak et Krieger 1912, Annal. Mycol. 10:52-53

≡ *Briosia cystopoides* (Bubak et Krieger) v. Arx 1972

= *Coremiella ulmariae* (MacWeeney) Mason apud Hughes 1953, Can. J. Bot. 31:640, fig. 94

≡ *Stysanus ulmariae* MacWeeney 1895, Irish Naturalist 4:277

non *Coremiella cuboidea* (Sacc. et Ellis) Ciferri et Caretta 1960

Because of the detailed account provided by Ellis (1971) only a brief description is given here. Arthroconidia (Fig. 5A) are alternate, separated from each other by one or more empty cells, smooth, thick walled, oblong or cubical with a small papilla at each end, brown, 3.5-7um x 4-9(14)um. Mature conidia are liberated by fracture or lysis of the outer hyphal wall of the adjacent empty cell often leaving a frill of outer wall material attached to the conidium. The fertile hyphae often show characteristic dichotomous branching (Bubak, 1912; Hughes, 1953). Conidiophores are loosely aggregated to form a coremium, hemispherical at the tip, rarely observed in culture.

Arnaud (1954) described and illustrated *Geotrichella alternata* without a Latin diagnosis. Though Kendrick and Carmichael (1973) suggest possible synonymy with *Coremiella* the status is not clear. Tubaki (in Kobayashi *et*. *al*., 1967) described *Geotrichella arctica*, a fungus bearing considerable resemblance to *Coremiella*. Unfortunately, it was not possible to obtain the type specimen for study.

The alternate arthroconidia of *Coremiella* distinguish this form-genus from dematiaceous fungi forming arthroconidia by fission such as *Scytalidium*, *Briosia* and *Bahusakala*.

In its conidium development *Coremiella* resembles both *Sporendonema* and *Malbranchea* but differs from both in having

darkly pigmented cell walls and also from the latter in the diameter of the fertile hyphae. The hyphae and arthroconidia of *Coremiella* are much wider 3.5-7um in diameter, whereas those of *Malbranchea* rarely exceed 3.5um.

## Habitat and Activities

This species has been recorded on senescent and living plant material and appears to have a worldwide distribution. Lucas, Carvalho and Barreiro (1974) report an incidence of soft rot in pears caused by *C. cubispora*.

*C. cubispora* digests cellophane but does not attack keratin. It grows vigorously at 30 C but fails to grow at 37 C.

On agar the fungus releases a pigment which appears grey or brown on cereal agar or yellow on PYE.

## Material Examined

UAMH 1165, photomicrograph of a DAOM specimen 35160, from *Ranunculus* leaf, England, determined by E.W. Mason; UAMH 1513, from *Picea abies*, Denmark by Mason 1949, from CMI as 36938; UAMH 3793, from stump of *Lindera umbellata* Japan by M. Ichinoe, 1966, received from Tubaki, IFO, Japan as 8862 (NHL 4583).

## *Geotrichum* Link 1809

Type Species

Geotrichum candidum Link 1809, Berlin Magazin 3:17; Persoon 1822, Mycologia Europaea 1:26; Carmichael 1957, Mycologia 49(6):820-830

For full synonymy refer to Carmichael (1957)

Perfect state:

Endomyces geotrichum Butler et Petersen 1972, Mycologia 64:367

The form-genus Geotrichum is distinguished by formation of hyaline arthroconidia in chains by segmentation of undifferentiated hyphae; conidiophores are absent. Arthroconidia develop by septation and disjunction at a double septum of lateral branches or the broader vegetative hyphae which may branch dichotomously. Arthroconidia are thin-walled, smooth, cylindrical or ellipsoidal or oblong, sometimes becoming subglobose.

The developmental sequence of arthroconidium formation in G. candidum and the Geotrichum state of Endomyces magnusii has been recorded by time lapse photography (Cole and Kendrick, 1969).

Numerous species have been described in Geotrichum making the taxonomy of the genus complicated and confused. For Geotrichum candidum alone, a species demonstrating considerable variation in culture, Carmichael (1957) lists

over 50 synonyms.

Furthermore, characters of many species included in *Geotrichum* are contrary to those attributed to the type species. For this reason, the following should be excluded from *Geotrichum*: dematiaceous species (see *Scytalidium*); fungi with differentiated conidiophores (see *Arthrographis* and *Oidiodendron*); species lacking fission arthroconidia (see *Coremiella*, *Sporendonema*, *Malbranchea* and *Oidiodendron*), and fungi forming blastoconidia as well as fission arthroconidia (see *Trichosporon*, *Moniliella*). Many species previously described in *Geotrichum* can be referred to one of these form-genera.

However, a number of difficult problems remain. In culture, the type species, *G. candidum*, appears dry, hairy or powdery, but the texture is slimy. This yeast-like texture is also characteristic of some other species of *Geotrichum*. If this yeast-like form of growth is a valid generic character, then fungi having dry-spored fission arthroconidia should be excluded. However, in order to exclude closely related fungi from *Geotrichum*, a suitable form-genus must be available for admission. As yet, no other form-genus has been proposed to accommodate non yeast-like fungi developing hyaline fission arthroconidia on undifferentiated hyphae. (See also arthroconidial states of Basidiomycotina.)

An additional problem, in assigning fungi to *Geotrichum*

is defining the degree of specialization of the conidiophore. According to the definition accepted by the Kananaskis Conference on Fungi Imperfecti (Kendrick, 1971, p. 224-228), the hypha of Geotrichum which fragments to form arthroconidia is a simple conidiophore, 'micronematous' of Ellis (1971). However, this fertile hypha is indistinguishable from the vegetative hypha before the process of conversion begins (Cole and Kendrick, 1969). As well, intercalary arthroconidia form in the broader vegetative hyphae (Carmichael, 1957). It is often difficult, therefore, to differentiate between an undifferentiated fertile hypha i.e. 'micronematous' conidiophore and a slightly differentiated i.e. 'semi-macronematous' (Ellis, 1971) conidiophore. Species with differentiated conidiophores are excluded from Geotrichum (see Arthrographis).

Without access to herbarium specimens or cultures, only a limited number of Geotrichum species could be reviewed. Where possible, the status of some has been determined from the available literature, but the study is by no means inclusive.

## Species

Geotrichum amycelicum Redaelli et Ciferri 1935, Archiv. fur Mikrobiologie 6:60

Von Arx (1972) suggested that this species, as well as

*G. gracile*, *G. vanryiae* and *G. hirtum* may belong in *Trichosporon*.

*Geotrichum fragrans* (Berkhout) Morenz 1960, Taxonomie und medizinische Bedeutung der zur Gattung *Geotrichum* Link gehorenden Arten p. 178 [= *Oospora fragrans* Berkh.]

*Geotrichum klebahnii* (Stantz) Morenz [as 'klebhani'], ibid, p. 180 [= *Oospora klebahni* Stantz]

*Geotrichum gracile* (Weigmann et Wolff) Windisch 1952, Beitr. Biol. Pflanzen 29:157

?= *Trichosporon* fide von Arx (1972)

*Geotrichum hirtum* Windisch 1952, Beitr. Biol. Pfanzen 29:157

?= *Trichosporon* fide von Arx (1972)

*Geotrichum* state of *Endomyces magnusii* Ludwig 1886, Ber. Deut. Bot. Ges. 4:17

For a description, refer to von Arx (1972).

*Geotrichum vanriji* Saez 1964, Bull. mens. Soc. linn. Lyon 33(7):266

Von Arx (1972) suggested *G. vanryiae* Saez is closely

related to *G*. *gracile* and *G*. *amycelicum* and all may belong in *Trichosporon*.

Excluded Species

*Geotrichum cinnamoneum* (Libert) Saccardo 1886, Michelia 2:636

Though I have not seen material, Saccardo's description and the study of Quinta (1968) suggests that this species may be identical to *Wallemia sebi* (Fr.) von Arx.

*Geotrichum cuboideum* (Sacc. et Ellis) Sumstine ≡ *Arthrographis cuboidea* q.v.

*Geotrichum dulcitum* (Berkh.) Windisch 1952, Beitr. Biol. Pflanz. 29:156

= *Protendomycopsis domschii* Windisch 1965, Beitr. Biol. Pflanz. 41:337 = *Protendomycopsis dulcita* (Berkh.) Gams et Domsch 1969, Nova Hedwigia 18:20

Although proposing a new combination, Gams and Domsch treat this species under *Trichosporon cutaneum*.

*Geotrichum flavo-brunneum* Miller *et al*. 1957 ≡ *Scytalidium flavo-brunneum* q.v.

*Geotrichum microsporum* Smith 1962 ≡ *Arthrographis cuboidea* q.v.

*Geotrichum purpurascens* (Bon.) Sacc. 1886 = *Sporendonema purpurascens* q.v.

*Geotrichum roseum* Grove 1886 ?= *Sporendonema purpurascens* q.v.

*Geotrichum rotundatum* (Cast.) Cif. et Red. 1935, Arch. Mikrobiol. 6:59 = *Trichosporon cutaneum* fide Lodder and Kreger van Rij according to Saez (1964)

*Geotrichum rugosum* (Cast.) Dodge 1935, Medical Mycology p. 219 = *Trichosporon cutaneum* fide Lodder and Kreger van Rij according to Saez (1964).

*Geotrichum suaveolens* (Lindner) Cif. apud Diddens et Lodder 1942, in Die anaskosporogen Hefen II:271 = *Moniliella suaveolens* (Lindner) von Arx 1972

## *Malbranchea* Saccardo 1882

See Part II

## *Oidiodendron* Robak 1932

Perfect States: *Myxotrichum* Kunze, *Byssoascus* von Arx

*Lectotype Species*

*Oidiodendron tenuissimum* (Peck) Hughes 1958, Can. J. Bot. 36:790

≡ *Periconia tenuissima* Peck 1893, N. Y. State Museum Report 46:113 (Basionym)

= *Oidiodendron fuscum* Robak 1932, Saetryk av. Nyt. Mag. Naturvidensk 71:251

This wood and soil fungus forms arthroconidia on branched or unbranched hyaline fertile hyphae borne at the apex of a pigmented conidiophore. Occasionally conidiophore production is suppressed with arthroconidia forming directly on undifferentiated fertile hyphae.

The exact mode of development of arthroconidia is not completely understood. Barron (1962) in his monograph of nine species reported that arthroconidia were formed either endogenously, remaining connected within the original outer hyphal wall until maturity, or more commonly, formed directly by segmentation of the hypha with each developing conidium drawing apart slightly but remaining connected by a gelatinous secretion between maturing conidia.

Cole and Kendrick (1969) in a time-lapse study of *O. truncatum* concurred with this latter view. They found that initial septation, possibly by double septa, was followed by rounding up and drawing apart of the immature arthroconidia

leaving a clear area between adjacent conidia. The clear areas, termed connectives, were often traversed by a septum. Cole and Kendrick (1969) agreed with Barron (1962) that the connectives were either remnants of the outer hyphal wall left after endogenous formation of conidia, or more probably, that they were gelatinous secretions from the maturing conidia. The presence of a septum within the connectives suggested that new terminal end walls distinct from the original septum were formed by the maturing arthroconidium. In a further discussion, Kendrick (1971, p. 162) designated this type of arthroconidium development as type 2.

Arthroconidia of *Oidiodendron*, separated from each other by gelatinous connectives are distinct from the fission arthroconidia of *Geotrichum* and *Arthrographis* and the alternate arthroconidia of *Malbranchea* and *Ovadendron*. *Oidiodendron* is further distinguished by its pigmented conidiophores.

For a discussion of the species of *Oidiodendron*, the reader is referred to Barron (1961, 1962), Morrall (1968), Cole and Kendrick (1969), Kobayasi *et*. *al*. (1969), Kiffer, Mangenot and Reisinger (1969) and Tokumasu (1973). In addition, *Oidiodendron* conidial states have been described for *Myxotrichum cancellatum* [= *Toxotrichum cancellatum*] (Orr and Kuehn, 1964a; Apinis, 1964), *Arachniotus flavoluteus* (Muller and Pacha-Aue, 1968) and *Byssoascus striatosporus* [=

Arachniotus striatosporus] (Barron and Booth, 1966; von Arx, 1971). However, the Oidiodendron conidial state of A. flavoluteus reported by Muller and Pacha-Aue (1968) appears doubtful since my examination of the type strain of A. flavoluteus (UAMH 3531, CBS 627.71, NRRL 1243) reveals no conidial state. In addition, neither Kuehn and Orr (1959), Udagawa (1963), nor von Arx (1971) have reported an asexual state for this species.

Oidiodendron state of Myxotrichum striatosporus

Perfect State:

Myxotrichum striatosporus (Barron and Booth) Sigler comb. nov.

≡ Arachniotus striatosporus Barron and Booth 1966, Can. J. Bot. 44:1060 (Basionym)

≡ Byssoascus striatosporus as 'striatisporus' (Barron and Booth) von Arx 1971, Persoonia 6(3):377

Barron and Booth (1966) described this species in Arachniotus having white confluent cleistothecia composed of narrow, hyaline peridial hyphae and fusiform, striate yellow ascospores. In their preliminary discussion, however, they noted that darkly pigmented hyphae occurred in association with the cleistothecia, but did not include their description in the Latin diagnosis. Von Arx (1971) removed the species from Arachniotus and proposed a new genus, Byssoascus, because of the fusiform, striate ascospores.

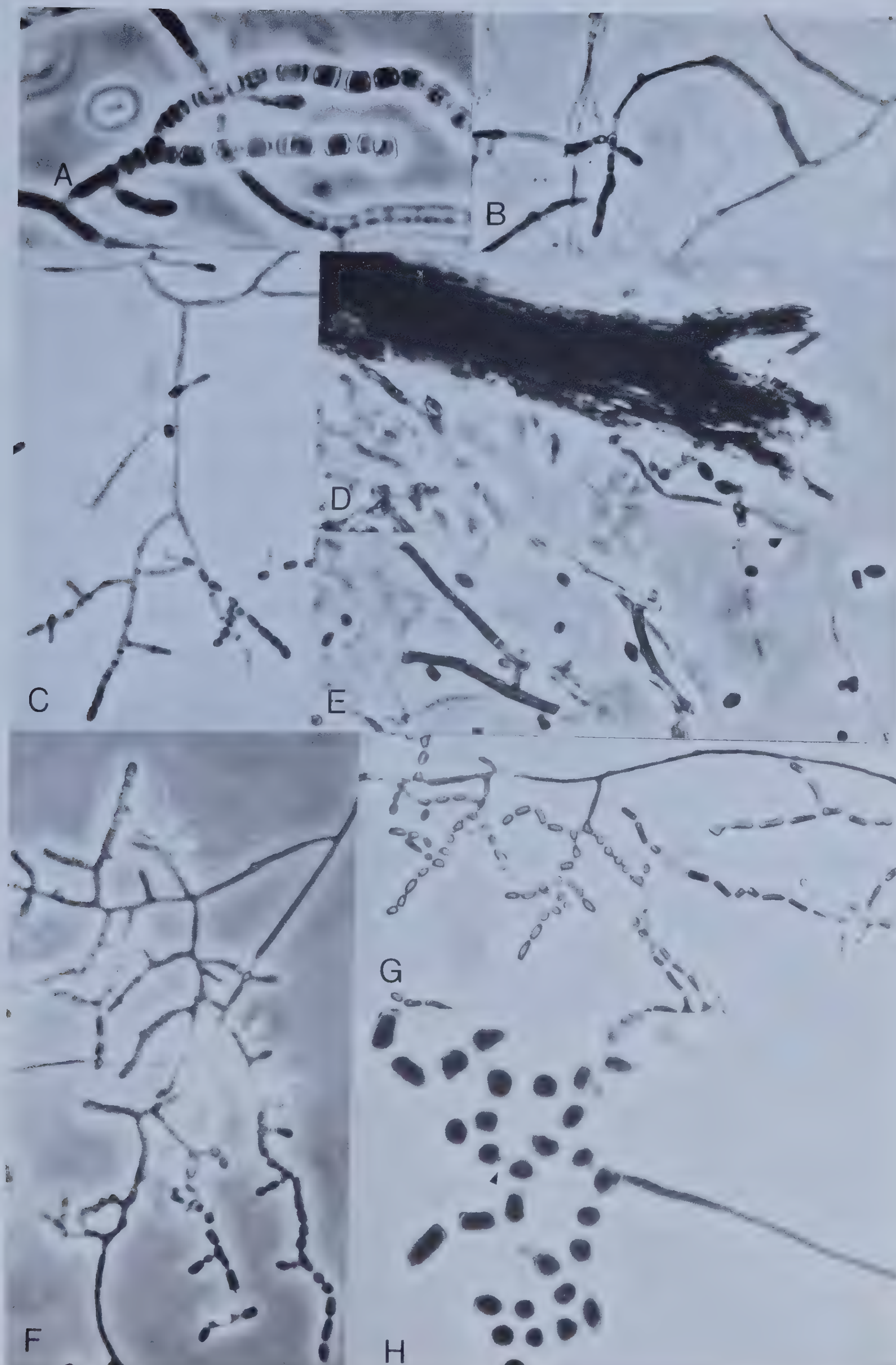
There appears to be little justification for creating a new genus for this fungus. On the basis of ascospore morphology alone, this species does not differ from species of _Myxotrichum_ which have ellipsoidal or fusiform ascospores. Ascospores of some species, such as _M. deflexum_ and _M. chartarum_ (Orr, Kuehn and Plunkett, 1963b; Apinis, 1964; Visset, 1974) also have longitudinal furrows which are clearly visible when the ascospore is seen in polar view at high magnification. The ascospores of _M. striatosporus_ are more definitely striate but this character would not exclude the species from _Myxotrichum_.

The primary reason for the removal to _Byssoascus_ would seem to be the nature of the cleistothecium, composed of thin walled narrow hyphae. Examination of the type strain (UAMH 3572 and 3758, CBS 642.66, CMI 115998) revealed that production of ascospores was consistently associated with the formation of dark brown, thick walled, smooth hyphae, often aggregated into ropes (Fig. 5D). When cultured on cereal agar, the colony is dense and the ascospores are produced beneath the surface among the thick walled hyphae. No discrete cleistothecium or peridial walls were seen. Other _Myxotrichum_ species have been described in which the cleistothecia coalesce and do not detach readily from the vegetative hyphae (see _Malbranchea circinata_, _Myxotrichum setosum_).

The transfer to _Myxotrichum_ is proposed for several

Figure 5. A. *Coremiella cubispora* (UAMH 3793). B-E. *Oidiodendron* state of *Myxotrichum striatosporus* (B,D,E,-3572; C-3578). F-H. *Oidiodendron* state of *Myxotrichum setosum* (3835). Fig. 5A. Alternate arthroconidia borne on dichotomously branched hyphae. Fig. 5B. Arthroconidia borne on pigmented conidiophore. Fig. 5C. Arthroconidia borne on hyaline conidiophore. Fig. 5D. Dark brown 'peridial' hyphae aggregated into ropes, and fusiform striate ascospores. Fig. 5E. Ascospores and segments of peridial hyphae showing blunt tipped branches. Figs. 5F-5G. Arthroconidia borne on hyaline conidiophores. Fig. 5H. Septum (arrow) traversing connective between adjacent arthroconidia. All x 830, except H, x 2300.

[illegible] state of *M[illegible]* *striatosporus* (B,D,E [illegible]) [illegible] H. *[illegible]* state of *Myxotrich[illegible]* [illegible] Fig. 5A. Alternate arthroc[illegible] [illegible] branched hyphae. Fig. 5B. [illegible] [illegible] conidiophore. Fig. 5C. [illegible] [illegible] conidiophore. Fig. 5D. Dark brown [illegible] [illegible]res. Fig. 5E. Ascospores and [illegible] [illegible] showing [illegible] tipped branches. Fig. 5F- [illegible] [illegible] borne on hyaline conidiophores. [illegible] [illegible] traversing connective between [illegible] [illegible] x 8[illegible], except H, x 2300.


A
B
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E
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G
H

reasons. First, the ascospore shape and surface, the *Oidiodendron* conidial state and the cellulolytic activity of the fungus are consistent with other species of *Myxotrichum*. In addition, the dark brown, thick walled, coalescing hyphae (Fig. 5D) are branched and often have blunt tips where the terminal portion has broken off (Fig. 5E). This severance of the tip of branches is seen frequently in species of *Myxotrichum* and is quite characteristic for the genus. Finally, the similarity of *M. striatosporus* to *M. setosum* suggests these two species should be retained in the same genus.

The *Oidiodendron* state of *M. striatosporus* is distinct and closely resembles the *O.* state of *M. setosum*. In culture on cereal agar, the conidiophores of *M. striatosporus* are mostly hyaline (Fig. 5C) and narrow in diameter, rarely becoming pigmented at the base (Fig. 5B). Often arthroconidia are formed directly on undifferentiated hyphae. Arthroconidia are smooth or slightly rough walled, olive green, barrel shaped 1.5-2.5um x 1.5-3.5um. Barron and Booth (1966) noted that the barrel shaped arthroconidia differed from arthroconidia of other species which were mostly ovoid, globose or cylindric.

In addition to the microscopic morphology, the colonial appearance of *M. striatosporus* and *M. setosum* is very similar. *M. striatosporus* grows slowly on both PYE and cereal. On cereal, the colony is 22 mm in diameter at 14

days, flat, slightly zonate, velvety, dark olive green with characteristic bright yellow margin, reverse brown. On PYE, the colony is pale olive green adhering poorly to the cellophane and lifting up at the margin.

Oidiodendron state of Myxotrichum setosum (Eidam) Orr and Plunkett, in Orr et. al. 1963b, Can. J. Bot. 41:1457-1480

?= Oidium microspermum Berkeley et Broome 1873, in Ann. Mag. Nat. Hist. 4, ser. II, 346

= Oospora microsperma (Berk. et Br.) Saccardo et Voglino 1886, Sylloge Fungorum 4:22

History

Berkeley and Broome (1873) described Oidium microspermum from bark of Scotch fir with a brief description but no illustration. Saccardo and Voglino (in Saccardo, 1886) transferred O. microspermum to Oospora based on Berkeley and Broome's description. Lindau (1907) described the fungus from an exsiccati specimen, Rabenhorst's Fungi europa 1577, from Lonicera xylosteum.

Since I have seen neither illustrations nor the type specimen of Oidium microspermum no new combination is proposed for the imperfect state. However, both specimens examined were identified by DAOM (37243 and 144716) as

*Oidium microspermum*. Therefore, it is listed as a probable synonym of the *Oidiodendron* state of *Myxotrichum setosum*.

According to Dale (1903), the perfect state, *Gymnoascus setosus* was first described by Eidam in 1882 at a meeting of the Schleisische Gesellschaft fur vaterlandische Cultur. The original description (Eidam, 1882) based on an isolate from a wasp's nest apparently was brief and lacked illustrations.

In 1902, Massee and Salmon in a study of coprophilous fungi, recovered *G. setosus* on a bee's nest in England. They provided a description and an illustration of their isolate (Figs. 6D,6E). Massee and Salmon noted the distinctive white centrum of the ascocarp.

Dale (1903) studied the isolate of Massee and Salmon in agar culture. However, the isolate failed to produce the sexual state in culture even after growth on a variety of media over a period of eighteen months. Dale reported a conidial state formed from the germinating ascospores, reproducing by budding when grown on 2% beer wort agar. However, after prolonged growth, vegetative hyphae formed which branched and bore conidia at the nodes. Dale noted the resemblance to conidium development in *Verticillium*.

DeLamater (1937) comparing *G. setosus* with *Eidamella spinosa* [= *Myxotrichum deflexum*] concluded the two species were distinct and disagreed with Dodge's (1935) placement of

_E_. _spinosa_ into synonymy with _G_. _setosus_. Apinis (1964) concurred with DeLamater.

Orr and Plunkett (in Orr _et_. _al_., 1963b) proposed a new combination _Myxotrichum_ _setosum_ (Eidam) based on the description and figures of Massee and Salmon (1902) and Dale (1903) and the original description of Eidam (1882). Orr and Plunkett were unable to find a type specimen nor any recent isolates of _G_. _setosus_. Despite the detailed account of Dale (1903) of the conidial state, Orr and Plunkett reported the asexual state to be unknown.

Apinis (1964) examined the original drawings of Massee (NY) of _M_. _setosum_ as well as two specimens from bee hives. In his description, Apinis (1964) described the conidial state as oidia, 2 - 3um in diameter.

Although an _Oidiodendron_ state has not previously been reported for _M_. _setosum_ it may have been overlooked. Conidiophore development is weak and conidiophores remain unpigmented. Arthroconidia often develop on undifferentiated hyphae. These are probably the oidia described by Apinis (1964). The conidial state of _M_. _setosum_ described by Dale (1903) is more difficult to interpret, though it is possible she was either working with a mixed culture, or misinterpreting the mode of conidium development as blastospores rather than arthrospores. The fact that she failed to find the perfect state in culture is not surprising since this species is psychrophilic. Its

common isolation from old nests of bees or wasps suggests that the sexual state is formed after an overwintering period.

Description

The colony on PYE at 25 C is slow growing, 17 mm in diameter at 21 days, pale olive, reverse tan, velvety, adhering poorly to the cellophane with the margin of the colony lifting up and becoming undulate and the center raised.

On cereal, the colony is flatter, stretching to 22 mm at 21 days with slightly raised center, powdery, dry and cracked, olive green in color with 1-2 mm wide pale yellow margin, buff reverse.

This species is psychrophilic, growing and forming the perfect state at 8 C.

Vegetative hyphae are hyaline, septate, narrow. Conidiophores (Figs. 5F,5G) are hyaline, narrow, 1-1.5um in diameter, 30-140um in length, branching at the apex to form fertile hyphae of the same diameter. Arthroconidia develop in more or less basipetal succession on the fertile branches (Fig. 5F) or on undifferentiated hyphae. Arthroconidia, becoming slightly rounded, remain linked by connectives often traversed by a septum (Fig. 5H). Arthroconidia are olive colored with thickened walls (Fig. 5H), smooth, mostly

cylindrical, sometimes subglobose or ovoid, 1.5-2um x 3-5um.

Homothallic. Cleistothecia confluent, not discrete. Peridial hyphae (Fig. 6A) not anastomosed, composed of dense, dark brown, septate, thick walled hyphae terminating in long spines (Fis. 6A,6D) bearing two or three short lateral spines, often opposite to each other (Fig. 6D). Ascomatal initials are coiled (Fig. 6B); asci are hyaline, evanescent, subglobose, 6-7um in diameter. Ascospores (Figs. 6C,6E,6F) are pale green at maturity, smooth, fusiform or navicular in shape, with one face flattened, 1.5-2um x 3.5-6.0um.

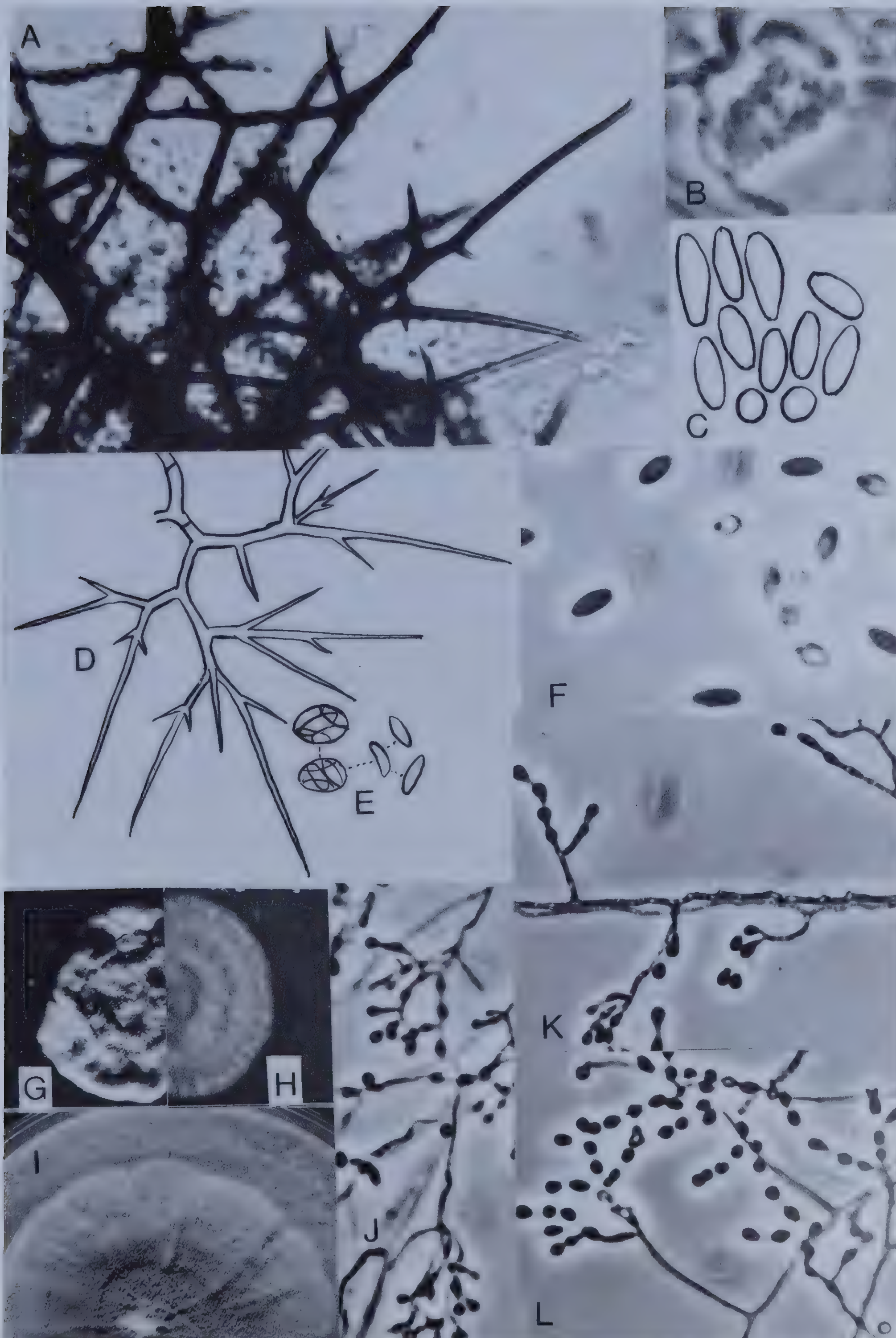
<u>Discussion</u>

The description and figures (Figs. 6D,6E) of Massee and Salmon (1902) correspond well with the fungus described here. In their description, they noted that the centrum containing asci remained permanently white. In young cultures, this is true, but as the ascospores mature, the centrum becomes greenish yellow in color.

The characteristic feature of <u>M</u>. <u>setosum</u> noted by Dale (1903) was the sharply pointed branched spines of the peridial hyphae which did not anastomose to form a discrete cleistothecium. The dark brown color of the peridial hyphae and the fusiform shape of the ascospores suggest that this species is correctly placed in <u>Myxotrichum</u>.

Figure 6. A-F. _Myxotrichum setosum_ (A-C,F-UAMH 3835). G-L. _Ovadendron asperulatum_ (G-J,L-183; K-2815). Fig. 6A. Coalescing dark brown sharply pointed spines of peridial hyphae, x 830 BF. Fig. 6B. Coiled ascomatal initials, x 2300. Fig. 6C. Ascospores, x 2700. Fig. 6D-6E. Illustration of Massee and Salmon (1902) showing peridial hyphae (x400) and ascospores (x 1000). Fig. 6F. Ascospores, x 2300. Figs. 6G-6I. Colonial morphology after 21 days at 25 C, x 1.0. G and H on PYE, I on cereal. Fig. 6J. Verticillately branched conidiophores bearing chains of arthroconidia, x 830. Figs. 6K-6L. Chains of arthroconidia, x 830.

[illegible] setosum (A-C, [illegible]; [illegible] (G-J, L-183; K-2815). [illegible] sharply pointed spines of [illegible], x [illegible]. Fig. 6B. Coiled ascomatal initial[illegible]. [illegible] Illustration of [illegible]ssee and Salmon (1902) showing [illegible] [illegible], x 2300. Figs. 6G-6I. Colonial morph[illegible] after [illegible] days at [illegible] C, x 1.0. G and H on PYE, I on [illegible]. Fig. 6J. Verticillately branched conidiophores [illegible] [illegible] of [illegible]conidia, x 830. Figs. 6K-6L. Chain[illegible] [illegible], x [illegible].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Placement of the conidial state in _Oidiodendron_ is most suitable for the present. Although the conidiophores are hyaline, the development of arthroconidia cannot be differentiated from other species of _Oidiodendron_, especially the _O._ state of _M. striatosporus_. However, if other similar fungi are discovered which lack the characteristic pigmented conidiophores of _Oidiodendron_ then a new form genus could be considered to accomodate these fungi. Indeed, the conidial state of the ascomycete, _Cookeina sulcipes_ (Berk.) Kuntze, was grown in culture and reported by Paden (1975) to form arthroconidia separated by connectives similar to the type described for _Oidiodendron truncatum_ by Kendrick (1971, p. 162). Paden (1975) did not describe the nature of the conidiophore.

## Habitat and Activities

Recorded from wood of _Picea glauca_, soil of Alpine regosol, Alberta, nests of wasps and bees, and honey-comb of bees, England and Germany.

This species is neither keratinolytic nor cellulolytic.

## Material Examined

UAMH 1174, photomicrograph of DAOM 37243, from _Picea glauca_; UAMH 3835, washed soil particles from Alpine regosol, Kananaskis, Alberta, between 1968 and 1972, received from DAOM as 144716 (Bissett, JB 922).

## *Oidium* Link 1809 = *Sporotrichum* Link 1809

*Type Species*

*Oidium aureum* (Pers.) Link 1809, Mag. Ges. Naturf. Freunde Berlin 3:18

= *Trichoderma aureum* Persoon 1801, Syn. Meth. Fung. p. 232

= *Sporotrichum aureum* Link 1809, Mag. Ges. Naturf. Freunde Berlin 3:13

Link proposed *O. aureum* for a specimen which he believed to be identical with *Trichoderma aureum* Persoon. However, the fungus which Link identified as *T. aureum* Persoon was in fact *T. dubium* of Persoon. Link, in the same paper, described several species of *Sporotrichum*, one of which was *S. aureum*, and Hughes (1958) recognized that *S. aureum* was identical with Persoon's *T. aureum*. Hughes therefore considered *Oidium* to be a synonym of *Sporotrichum*.

Although Hughes (1958) and others (Carmichael, 1962; von Arx, 1970; Ellis, 1971; Kendrick and Carmichael, 1973) have rejected *Oidium*, the name is still widely used, both for lignicolous Hyphomycetes which have their perfect state in *Botryobasidium* (Pouzar and Jechova, 1967; Holubova-Jechova, 1969; Eriksson and Ryvarden, 1973), and for conidial states of Erysiphales. Kendrick and Carmichael

(1973) referred species of the first group to *Alysidium*, *Olpitrichum* and *Acladium* and species of the latter group to *Acrosporium*.

Recently, Weresub (1973) discussed the controversy over *Oidium* and agreed with Hughes' invalidation of *Oidium*. However, because of the widespread use of the name by both plant pathologists, for the conidial states of Erysiphales, and basidiomycete taxonomists for the conidial states of *Botryobasidium*, she proposed that a decision be made for conservation of *Oidium* for one or other group. However, she recommended that if both proposals were rejected, the use of *Oidium* should be discontinued.

Other Species

*Oidium microspermum* Berkeley et Broome 1873, in Ann. Mag. Nat. Hist., ser. II, 4:346

?= *Oidiodendron* state of *Myxotrichum setosum* q.v.

## *Oospora* Wallroth 1833

Type Species

Eleven original species, including the type species of four other genera were included in *Oospora* by Wallroth (Fl. Crypt. Germ. 2:182, 1833)

Numerous species have since been added to *Oospora*, a

nomen illegitimum, according to Hughes (1958). Some *Malbranchea* species may have been described previously in *Oospora* but in most cases, the descriptions, often lacking illustrations, are inadequate for an accurate comparison. Examination of the type specimen, if still available, would be required to determine the correct relationships.

## Species

*Oospora crustacea* Saccardo 1882, Michelia 2:545 ?= *Sporendonema casei* q.v.

*Oospora cuboidea* Saccardo et Ellis 1882, Michelia 2:576 = *Arthrographis cuboidea* q.v.

*Oospora microsperma* (Berk. et Br.) Saccardo et Voglino 1886, Syll. Fung. 4:22 ?= *Oidiodendron* state of *Myxotrichum setosum* q.v.

# *Ovadendron* Sigler et Carmichael gen. nov.

## Diagnosis

Vegetative hyphae narrow, hyaline, septate. Conidiophores, if present, are narrow, hyaline, branched verticillately at the apex, at an acute angle. The fertile branches are narrow, at first sparingly, then more regularly septate. Aleurioconidia, formed terminally or laterally on

short pedicels, intergrade with alternate arthroconidia. Alternate arthroconidia, broader in diameter than the fertile hypha, develop in long chains and predominate in some species. Mature conidia, released by lysis of intervening segments are smooth or roughened, hyaline or yellow, barrel shaped, cuneiform, subglobose or pyriform. Type species: *Ovadendron asperulatum* Sigler et Carmichael sp. nov.

## Discussion

*Ovadendron* differs from *Malbranchea* in forming arthroconidia which are broader than the diameter of the fertile hypha. The conidiophores of *Ovadendron* distinguish this genus from *Chrysosporium* in which aleurioconidia form directly on the sides of the hypha or on short pedicels, and rarely develop in chains of more than two or three conidia.

*Ovadendron asperulatum* Sigler et Carmichael sp. nov.

## Description

Colonies on PYE (Fig. 6G) growing moderately rapidly (35-39 mm in 21 days) are first pale pink, flat and cottony, becoming vivid mustard yellow, with pink and white patches at the periphery, powdery, reverse tan. The colony adheres poorly to the cellophane, lifting in the center with few radial folds, and curling up at the margin with new growth

appearing below. Poorly sporulating colonies (Fig. 6H) are flatter, pale yellow in color with hairy or bristly surface.

On cereal, colonies (Fig. 6I) grow faster (55 mm in 21 days) and are flat, with outward radiating folds, at first white then mustard yellow, reverse the same, powdery. As sporulation occurs, the center (Fig. 6I) becomes flatter than the outer periphery. A brown diffusing pigment appears below the colony by 14 days.

Growth is poor at 30 C and no growth occurs at 37 C. On oatmeal agar at 18 C and 25 C a dark brown pigment diffuses into the agar.

The vegetative hyphae are hyaline or yellow, narrow, septate. Conidiophores are narrow, 0.5-1.0um in diameter, 20-150um in length, hyaline, sometimes branching along the length, verticillately near the apex (Fig. 6J). Fertile hyphae are narrow, 0.5-1.0um in diameter, at first sparingly then more regularly septate, with concentration of cytoplasm in alternate segments. Arthroconidia (Figs. 6K,6L) become thick walled and increase in volume, maturing in long chains in more or less basipetal succession. Mature arthroconida (Fig. 6L), released by lysis of the intervening empty cell, are yellow, slightly roughened, barrel shaped or cuneiform if terminal, 1.5-2.5(3)um x 2.5-4.5(5)um.

Holotype: soil, Harvard Forest, Massachusetts, by F. Raymond (II-1-9), UAMH 183

Habitat and Activities

*Ovadendron asperulatum* has been recorded from soil in Massachusetts, Ontario and Costa Rica.

It is strongly cellulolytic but does not attack keratin.

Material Examined

UAMH 182, soil, Harvard Forest, Mass., by F. Raymond, Farlow Herbarium as III-4-1; UAMH 183, TYPE, soil, Harvard Forest, Mass., by F. Raymond, as II-1-9; UAMH 2169, Puerto Vieho, Costa Rica, from G.L. Barron, Univ. of Guelph as 10258; UAMH 2815, white pine soil, St. William, Ontario, by G. H. Bhatt, 1967, as U.W. 316.

*Ovadendron sulphurea-ochracea* (v. Beyma) Sigler comb. nov.
= *Oospora sulphurea-ochracea* van Beyma thoe Kingma 1933, Zentralbl. f. Bakter. u. Parasitenkde, 2 Abt., 88:134 (Basionym)

Description

The colony grows moderately rapidly (30 mm in 14 days) and is white, reverse tan, downy in the center and slightly raised with outer glabrous margin. On cereal, the colony is flat (35 mm in diameter at 14 days), white, reverse tan, floccose.

Vegetative hyphae are hyaline, septate, narrow. Fertile hyphae arise as narrow, 0.5-1.0um in diameter, short, coiled, lateral branches (Figs. 7A,7B) becoming regularly septate, with concentration of cytoplasm in alternate segments. The arthroconidia become thick walled, and increase in volume, maturing in more or less basipetal succession. Released by dissolution of the alternate empty segments, arthroconidia are barrel shaped, hyaline, smooth 2-2.5um x 2.5-4um.

## Habitat and Activities

The original isolate of van Beyma was from sputum, Holland.

*O. sulphurea-ochracea* is keratinolytic, producing moderate digestion and penetration of hairs by single hyphae. Cellophane is not attacked.

## Material Examined

UAMH 181, ? TYPE strain, from CBS as strain van Beyma received in 1954, and as CBS 233.32 received in 1975.

*Ovadendron pannorum* (Link) Sigler et Carmichael comb. nov.

= *Sporotrichum pannorum* Link 1824, Linn. Spec. Plant. IV, 6(1):13 (Basionym)

≡ <u>Chrysosporium</u> <u>pannorum</u> (Link) Hughes 1958,
Can. J. Bot. 36:749

= <u>Geomyces</u> <u>vinaceus</u> Dal Vesco 1957, Allionia 3:14

Perfect State:

<u>Pseudogymnoascus</u> <u>roseus</u> Raillo 1929, Zentralbl. Bakt. Parasitenkde, Abt. 2, 78:520, fig. 2

= <u>Gymnoascus</u> <u>rhousiogongylinus</u> Wener and Cain 1970, Can. J. Bot. 48:325

<u>History</u>

For full synonymy and description of <u>O</u>. <u>pannorum</u> refer to Carmichael (1962). Though we have not seen the description of <u>Geomyces</u> <u>vinaceus</u>, Samson (1972) stated that the <u>Chrysosporium</u> conidial state of <u>P</u>. <u>roseus</u> was described as <u>Geomyces</u> <u>vinaceus</u> by Dal Vesco. Also, our strain (UAMH 1643) received from H. H. Kuehn as <u>G</u>. <u>vinaceus</u> Dal Vesco is <u>O</u>. <u>pannorum</u>.

<u>Description</u>

<u>O</u>. <u>pannorum</u> is distinguished by narrow, 0.5-1.0um in diameter, hyaline, 10-100um in length, conidiophores branching verticillately near the apex at an acute angle (Figs. 7C,7D). Aleurioconidia formed terminally or laterally on short pedicels intergrade with intercalary arthroconidia (Fig. 7D). Mature conidia are cuneiform,

subglobose or pyriform or barrel shaped if intercalary, hyaline, smooth or roughened, 2-4um x 2-5um, mostly 2 x 3um.

## Discussion

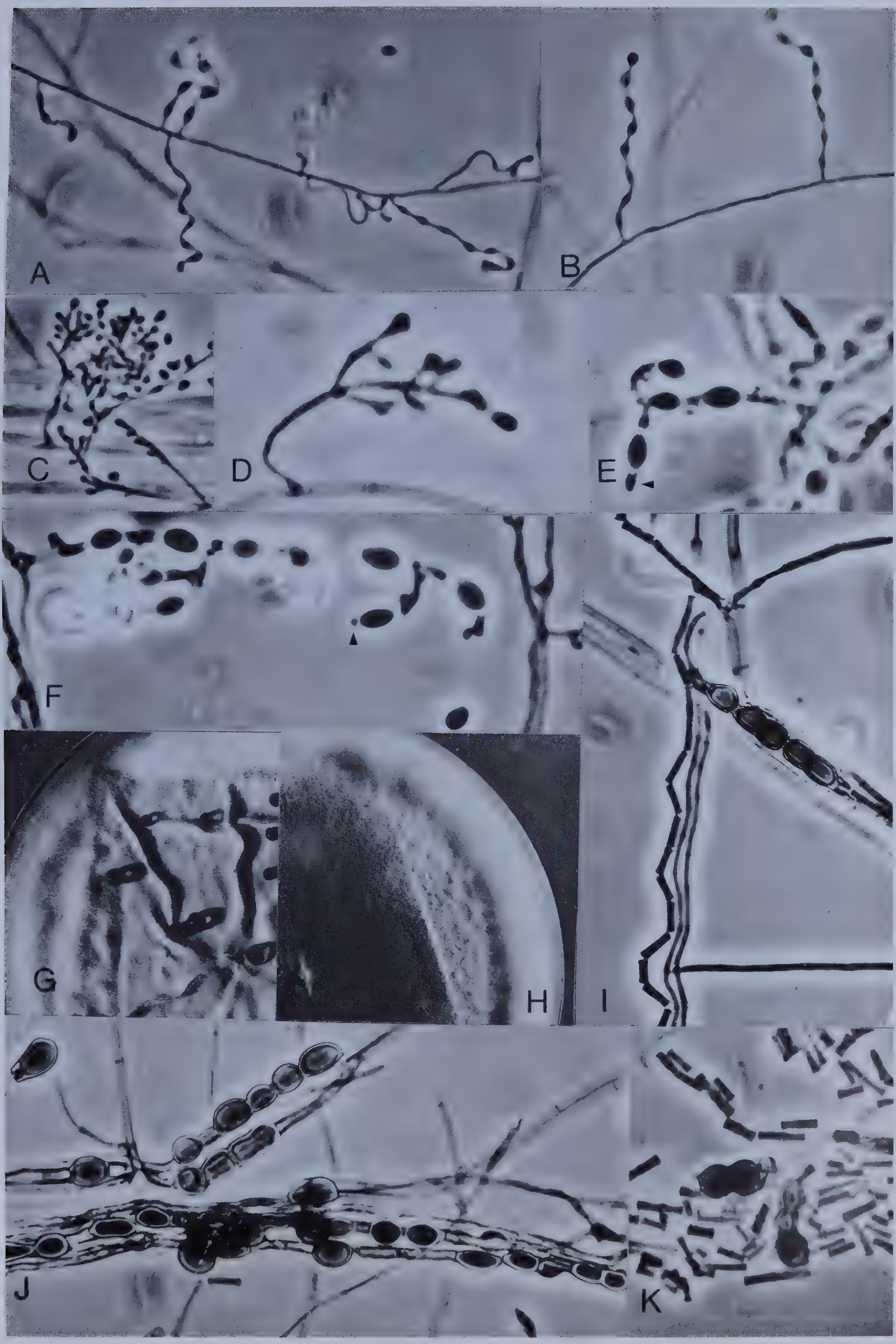
O. pannorum differs from O. asperulatum in forming conidia in short chains of 2 to 4, and in forming aleurioconidia laterally on the hypha.

Considerable variation in colonial morphology is apparent in strains of O. pannorum. However, the perfect state, P. roseus, is associated only with isolates having purplish-red colored colonies. Cleistothecia form readily on oatmeal agar at 18 C. It appears likely that O. pannorum is a complex composed of more than one species; however, the uniform microscopic morphology precludes separation into additional species for the present.

One of the strains examined (UAMH 3775, CBS 298.49) was received from the CBS as Onygena piligena, isolated from woollen slipper by R. Heim. The microscopic and colonial morphology of this strain conforms to O. pannorum. The relationship between O. pannorum and Onygena piligena is uncertain, though presumably this strain was isolated from ascocarps found on the natural substrate. However, no ascocarps have been produced by this strain in culture. The ascocarps of Onygena are notoriously difficult to reproduce on artificial media, though Tubaki (1960) induced ascocarp formation in O. corvina by growing it on a medium enriched

Figure 7. A-B. _Ovadendron sulphurea-ochracea_ (UAMH 181a). C-D. _Ovadendron pannorum_ (3775). E-F. _Ptychogaster albus_ (3813). G-J. _Scytalidium lignicola_ (1502). K. _Scytalidium album_ (3620). Figs. 7A-7B. Arthroconidia in chains on coiled lateral branches. Figs. 7C-7D. Aleurioconidia and arthroconidia borne on verticillately branched conidiophores. Figs. 7E-7F. Chains of conidia developing serially (arrow) and forming clamp connections between each conidium. Figs. 7G-7H. Colonial morphology after 21 days at 25 C. G on PYE, H on cereal. Fig. 7I. Hyaline arthroconidia formed by fragmentation of undifferentiated hypha. Fig. 7J. Chains of thick walled brown arthroconidia. Fig. 7K. Mature arthroconidia of both types. Colonies x 1.0, others x 830, except D, x 2300.

Figure 7. A-B. Ovadendron [illegible]. C-D. Ovadendron pannorum (3775). [illegible] (3813). G-J. Scytalidium lignicola (7502). K. [illegible] Figs. 7A-7B. [illegible] Figs. [illegible] branched conidiophores. Figs. 7E-7F. [illegible] developing serially (arrow) and forming clamp c[illegible] between each conidium. Figs. 7G-7H. Colonial [illegible] after 21 days at 25 C. G on PYE, H on cereal. Fig. 7I [illegible] Fig. [illegible] brown arthroconidia. Fig. 7K. Mature arthroconidia [illegible]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with owl's claw or human fingernail. Difficulties are also encountered in inducing initial ascospore germination (Tubaki, 1960).

The cellulolytic activity of this strain (UAMH 3775) suggests that this isolate was probably a contaminant as _Onygena_ is strongly keratinolytic (Tubaki, 1960).

## Habitat and Activities

_O. pannorum_ is ubiquitous and has a world wide distribution (Carmichael, 1962; Williams and Pugh, 1974).

This species is cellulolytic. _O. pannorum_ does not digest keratin, but some strains penetrate the hair by single hyphae.

## Material Examined

UAMH 650, plate contaminant, 1959; UAMH 1561, blood transfusion flask, 1962, from de Vries, CBS as 117; UAMH 1643, as _Geomyces vinaceus_, 1957, from Kuehn, Medford, New Jersey as 1; UAMH 1860, soil, Dugway, from Orr as 10 DM; UAMH 3775, woollen slipper, R. Heim, from CBS as 298.49 (_Onygena piligena_);

Material of _Pseudogymnoascus roseus_: UAMH 1644, ? TYPE strain of _Pseudogymnoascus vinaceus_, by Dal Vesco, Italy, from H. Kuehn, Medford, N.J. as 2; UAMH 1736, same strain as 1644 from Orr as 'P.g. Dal Vesco'; UAMH 1990, soil, Wisconsin, from Orr as QM 6969; UAMH 2005, soil, Japan, from

Orr as NHL 2284; UAMH 2879, soil under _Pinus contorta_, Kananaskis, Alberta, by P. Widden, 1967, from G. Bhatt, U. Calgary, Alberta, as 70 (=CBS 387.69); UAMH 3001 and 3002, from R.A. Morrall, Univ. Saskatchewan, as RM 2509 (SSF176) and RM 2169 respectively; UAMH 3166, mouse dung, Claremount, California, by R.K. Benjamin, 1965, as 1558; UAMH 3337, TYPE of _Gymnoascus rhousiogongylinus_, forest soil, Parry Sound, Ontario, by H.M. Wener, 1970, from Cain, U. Toronto Herbarium, as TRTC 45536 (CBS 722.69); UAMH 3875, Orr (O-3729)

## _Ptychogaster_ Corda 1838

_Type Species_

_Ptychogaster albus_ Corda 1838, Icones Fungorum II:23-24, Taf. XII, fig. 90

= _Ceriomyces albus_ (Corda) Saccardo 1888, Sylloge Fungorum 6:388

= _Ptychogaster fuliginoides_ (Pers. ex Steudel) Donk 1972, Proc. K. Ned. Akad. Wet. C 75:165-177

Perfect State:

_Tyromyces ptychogaster_ (Ludwig) Donk 1933, Mededeelingen Botanisch Museum Herbarium Rijks Universiteit 22:153

= _Polyporus ptychogaster_ Ludwig 1880, Zeitschr. ges. Naturwiss. III, 5:424-431

= *Oligoporus ustilaginoides* Brefeld 1889, Untersuchungen aus dem Gesammtgebiete der Mykologie, Heft 8:114-142, Taf. VII, fig. 23-25 and Taf. VIII, fig. 26-33

According to von Arx (1973), the fungus described by Corda as *Ptychogaster albus* was transferred by Saccardo (1888) to *Ceriomyces*. Brefeld (1889), including it in *Oligoporus* as *O. ustilaginoides*, provided excellent illustrations.

Illustrations by Brefeld (1889) of two species *O. ustilaginoides* and *O. farinosus* are similar and closely resemble an isolate (UAMH 3813, WFPL 170AD) received as *Ptychogaster albus*, from *Picea*, Nova Scotia.

Development of conidia in *Ptychogaster albus* resembles *Arcuadendron*. Conidia are formed in chains, with the conidiogenous cells occurring serially, developing from the apex of the newly formed conidium (Figs. 7E,7F). *Ptychogaster albus* differs, however, in forming clamp connections between each developing conidium (Fig. 7E). Mature conidia are yellow, smooth, ellipsoidal, ovoidal or barrel shaped, 4-5um x 6-9um, released by lysis of the intervening hypha.

Conidia of *Ptychogaster rubescens* Boudier develop in the same manner. For a description of *P. rubescens*, refer to Fidalgo (1958) and von Arx (1973).

*P. albus* and other species of *Ptychogaster* are conidial

states of polypores. In nature, the imperfect *Ptychogaster* stage grows as cushion shaped sporophores resembling conks, or small pads, composed of hyphae and powdery masses of conidia, or grows within the basidiocarp (Corda, 1838; Brefeld, 1889; Rea, 1922 p. 660; Donk, 1933; Davidson, Campbell and Weber, 1942; Davidson, Christensen and Darley, 1945; Fidalgo, 1958; Singh, Singh and Bakshi, 1961).

## Habitat

On wood of various types including *Pinus*, *Abies*, *Picea*, and mine timber wood, South Africa, Germany, Canada, India and the USA.

## *Scytalidium* Pesante 1957

## Generic Description

The form-genus *Scytalidium* is characterized by dematiaceous intercalary or terminal arthroconidia formed by fragmentation of undifferentiated hyphae. The arthroconidia are thick-walled, smooth, occasionally verrucose in age, 0-1 septate, pale to mid-brown, or yellowish brown, cylindrical, oblong or broadly ellipsoidal, and if septate, often constricted at the septum. Fission arthroconidia of a second type are hyaline, thin walled, smooth, cylindrical, single celled. (Also refer to Ellis, 1971.)

In its thallic ontogeny, *Scytalidium* most closely

resembles <u>Geotrichum</u> but is distinguished by its dematiaceous appearance.

KEY TO THE SPECIES OF <u>Scytalidium</u> ................ Table II

<u>Type Species</u>

<u>Scytalidium lignicola</u> Pesante 1957, Annali de la sperimentazione Agraria (Roma) II, suppl. CCLXI-CCLXV; Ellis 1971, Dematiaceous Hyphomycetes p. 28, fig. 2

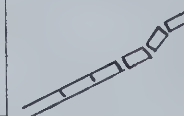
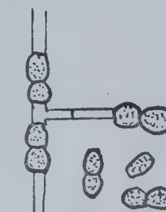
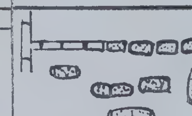
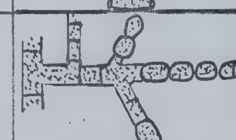
<u>Description</u>

Growth on PYE (Fig. 7G) is rapid reaching a diameter of 70 mm or filling the petri dish by 14 days. At first white, the colony turns dark tan, pale grey or dark grey in color, front and reverse, and is flat with raised radial folds, downy or wooly, dense, matted. Brown or black pigment is excreted into the agar. In addition the type culture (UAMH 1506) forms large dark brown exudate droplets on the surface.

On cereal agar (Fig. 7H), growth is slightly more rapid (80-90 mm in 14 days) but the aerial growth is scant. Colonies at first are flat, glabrous with reddish tan surface growth, gradually turning black as sporulation commences and with a loose weft of white cottony aerial growth. The agar turns plum or grey in color from diffused pigment. UAMH 2816 differs in growing faster than the type

TABLE II

KEY TO THE SPECIES OF SCYTALIDIUM

| Species | Growth Rate | Colony Color | Pigment | Activity | Growth 37 C | Arthroconidia Size | Septa | Color | Figure |
|---|---|---|---|---|---|---|---|---|---|
| Scytalidium lignicola | Rapid | Tan Grey Black | Brown Plum | C | – | 2 x 4.5-8 um<br>4-7 x 7.5-17um | 0<br>0-1 | Hyaline<br>Yellow-Brown |  |
| Scytalidium album | Rapid | White Grey | Yellow Grey | C | – | 2 x 5-8um<br>4.5-6(9) x 6.5-14-16um | 0<br>0-1 | Hyaline<br>Yellow-Brown |  |
| Scytalidium aurantiacum | Rapid | Yellow | Yellow Orange | C | – | 2 x 3.5-6um<br>4.5 x 8-13.5um | 0<br>0-1 | Hyaline<br>Yellow-Brown | |
| Scytalidium acidophilum | Slow | Grey-Brown | – | C | Scant | 4.5-6.5(8) x 7-23um | 0-1 | Brown |  |
| Scytalidium flavobrunneum | Rapid | Peach Grey | Yellow | C | – | 3.5-5 x 5.5-12(22) um | 0-1 | Brown Refractile Apices |  |
| Scytalidium state of Hendersonula toruloidea | Rapid | Grey Black | Grey | C K? | ? | 3.5-5 x 6.5-15um | 0-1 | Brown |  |

C Cellulolytic — Absent
K Keratinolytic

culture (UAMH 1506) and in forming dense black colonies resembling a coating of thick tar on the cellophane membrane.

There is no growth at 37 C.

Hyphae septate, hyaline, becoming brown. Arthroconidia of one type, formed by fragmentation of hyaline hyphae (Fig. 7I) are cylindrical, thin-walled 2um x 4.5-8um. Arthroconidia of the second type (Fig. 7J) arising in chains in an intercalary or terminal position on short, lateral branches, become thick walled, yellowish brown, broadly ellipsoidal, or oblong or barrel shaped, 0-1 septate, constricted at the septum, and show an increase in volume. Arthroconidia measure 4-7um in width and 7.5-12um in length if 0-septate and 12-17um if 1-septate. Arthroconidia are not readily detached and may appear surrounded by a brown slime.

## Habitat and Activities

Isolated from blue stain of wood of *Platanus*, *Pinus*, *Picea*, *Abies*, *Betula*, *Populus* and *Arachis*, and from soil and roots of *Vitis*, in Italy, Sweden, southern USA, Canada, Japan, Cyprus, England, India, Rhodesia (Shields, 1969; Ellis, 1971; Murao, Oda and Matsushita, 1973; Klingstrom and Johanssen, 1973) and also from rhizosphere of *Trifolium alexandrinum*, Pakistan (Hussain and Malik, 1972).

*S. lignicola* digests cellophane slowly, but fails to penetrate hair.

Klingstrom and Johanssen (1973) testing the antagonistic properties of several isolates of *S. lignicola* against *Fomes annosus*, *Polyporus versicolor*, *Lentinus lepideus*, and *Coniophora puteana* found *S. lignicola* overgrew all isolates, in the process killing the decay fungi.

*Material Examined*

UAMH 385, photomicrographs of DAOM 59090, from presumed type; UAMH 1502, TYPE, from *Platanus* wood, Italy, by Pesante and Peyronel, 1956, received from CMI as 62532 (CBS 233.57); UAMH 2816, from cornfield soil, Bright, Ontario, by G.C. Bhatt, 1967, as U.W. 655.

Two other species, *Scytalidium album* Beyer and Klingstrom and *S. aurantiacum* Klingstrom and Beyer having morphological and physiological differences from *S. lignicola* have been described. Microscopically, it is difficult to differentiate among the three species, the size of the arthroconidia of both *S. album* (Fig. 7K) and *S. aurantiacum* falling within the range for *S. lignicola*.

*Scytalidium album* Beyer and Klingstrom 1965, Svensk Botanisk Tidskrift 59(1):35

Colonies grow rapidly (50 mm on PYE and 70 mm on cereal at 14 days) and are white, flat, downy or glabrous. As

sporulation begins, the surface growth on cereal turns grey, and a grey pigment diffuses into the medium, with an orange pigment forming near the center. On DSA the pigment turns the agar bright lemon yellow.

Klingstrom and Beyer (1965) and Klingstrom and Johanssen (1973) distinguish _S. album_ from _S. lignicola_ by its moderate growth at 25 C, and its inability to grow at 35 C, by its whitish colored colonies and pale yellow pigmentation on malt agar, and its antagonistic properties.

_Habitat and Activities_

Occurrence: Isolated from blue stain of wood of _Betula_, _Acer_, _Populus_, _Pinus_, _Abies_, _Picea_, and _Pseudotsuga_ (Douglas fir) utility poles, in Sweden, Finland, eastern Canada and western USA (Klingstrom and Beyer, 1965; Ricard and Bollen, 1968; Klingstrom and Johanssen, 1973).

Antibiotic Activity and Metabolites: Klingstrom and Johanssen (1973) studying the action of _S. album_ against four decay fungi found inhibition resulted from a soluble substance excreted into the medium. Production of a yellow pigment was associated with antagonistic ability, but the pigment itself had no antibiotic activity. The active agent was extracted from culture filtrates of _S. album_ and found to be inhibitory to _Fomes annosus_.

One of the _S. album_ strains studied by Klingstrom and

Johanssen (1973) was the FY strain (UAMH 3620) of Ricard and Bollen (1968). Considerable interest has been generated in the metabolites produced by this strain. Ricard and Bollen (1968) reported inhibition of the wood decay fungus _Poria carbonica_ and some bacteria by culture filtrates. Stillwell, Wall and Strunz (1973) isolated and defined the structure of scytalidin, a second antiobiotic compound active against a wide variety of fungi. In addition to scytalidin, other antibiotic agents were present in culture filtrates, active against some fungi where scytalidin was not.

Findlay and Kwan (1973 a,b) defined another major metabolite, scytalone (3,6,8 -trihydroxytetralone) and a minor one (6,4,8 -dihydroxytetralone), neither of which were inhibitory to any significant degree. Similarly, Geigert, Stermitz and Schroeder (1973) isolated two other compounds, hexenophenones, neither inhibitory to _Poria carbonica_.

Activity: _S. album_ is cellulolytic, digesting cellophane after prolonged growth. It does not attack hair.

_Material Examined_

UAMH 3611, TYPE, from Norway spruce at Garpenberg, Sweden by Klingstrom and Beyer, 1963 (FF28), received from CBS as 372.65; UAMH 3620, from heartwood of Douglas fir utility poles, western Oregon, by Ricard and Bollen (FY), received from Wang, N.Y. State University, Syracuse as 1041.

*Scytalidium aurantiacum* Klingstrom and Beyer 1965, Svensk Bot. Tidskr. 59(1):35

Colonies grow moderately rapidly (40 mm on PYE and 65 mm on cereal at 14 days). On PYE colonies are pale yellow, flat with a woolly matted texture appearing coarse or bristly. A lemon yellow pigment diffuses into the agar on PYE and DSA.

Klingstrom and Beyer (1965) and Klingstrom and Johanssen (1973) distinguish *S. aurantiacum* from *S. lignicola* by its slower growth at 25 C and its inability to grow at 35 C. *S. aurantiacum* differs from *S. album* in the yellow-red color of the colonies and visible spots apparent on malt agar. *S. aurantiacum* was found to be antagonistic to some human pathogenic bacteria and to a number of wood decay fungi. Three isolates studied by Klingstrom and Johanssen (1973) were lethal for the decay fungi *Fomes annosus*, *Polyporus versicolor*, *Lentinus lepideus* and *Coniophora puteana*.

## Habitat and Activities

Isolated from pulpwood of *Pinus*, *Betula* and *Picea* in Sweden (Klingstrom and Johanssen, 1973).

*S. aurantiacum* is cellulolytic weakening the cellophane membrane after prolonged growth.

The single isolate examined penetrated hairs by single hyphae with no visible digestion of the hair.

Material Examined

UAMH 3612, TYPE, from pulpwood of *Pinus silvestris* at Skinnskatteberg, Sweden, by Klingstrom and Beyer (FF21), 1962, received from CBS as 374.65.

*Scytalidium acidophilum* Sigler et Carmichael 1974, Can. J. Microbiol. 20(2):267-268

Arthroconidia formed in extended chains (Fig. 8A), not easily detached, are brown, thick walled, smooth or verrucose in age, 0-1 septate, measuring 4.5-6.5(8) x 7-13(16)um if single celled and 4.5-6.5(8) x (10)11.5-23um if two celled. In shape, mature arthroconidia are broadly ellipsoidal, cylindrical or irregulary shaped, and if septate, constricted at the septum.

Sporulation and growth is enhanced on acid medium. Colonies grow slowly (21-26 mm in 21 days on cereal agar) and are dark grey brown, reverse dark grey, furrowed, cracked with a thin velvety nap. There is scant growth at 37 C.

Habitat and Activities

Isolated from acid soil near sulfur stockpiles, uranium

mine drainage water, and acid solutions containing 4% copper sulfate from an industrial plant, Canada and U.S.A. (Sigler and Carmichael, 1974; Starkey and Waksman, 1943).

*S. acidophilum* is tolerant to extreme acidity and high concentrations of copper (Starkey and Waksman, 1943; Starkey, 1973; Gould, Fujikawa and Cook, 1974). Sensitivity to copper increases as the pH approaches neutrality (Starkey, 1973).

*S. acidophilum* neither attacks cellophane nor digests hair.

Material Examined

From soil near sulfur pile, Bowden, Alberta: UAMH 3460 (CMI 183518, DAOM 145649, CBS 270.74, ATCC 26772) TYPE, November 1971; UAMH 3492, 3493, 3494 (CMI 183519, CBS 271.74, ATCC 26773), June 1972; From liquid: UAMH 3489 (CMI 173066, CBS 421.73, ATCC 24569) from industrial plant acid solutions, 'Fungus D' of Starkey and Waksman (1943); UAMH 3535 (CMI 183521, CBS 272.74, ATCC 26774) from uranium mine drainage water, Ivarson, 1972.

*Scytalidium flavo-brunneum* (Miller, Giddens et Foster) Sigler comb. nov.

= *Geotrichum flavo-brunneum* Miller, Giddens, et Foster 1957, Mycologia 49:792, figs. 5-7 (Basionym)

This species forms brown arthroconidia in chains on

lateral branches (Figs. 8B,8C); conidiophores are absent. Fertile branches formed on hyaline vegetative hyphae, become regularly septate with a concentration of cytoplasm. At disjunction the conidia (Fig. 8D) are cylindrical, truncate or oblong, expanded in volume, brown with refractile hyaline apices (Figs. 8D,8E) predominately single celled and measure 3.5-5um x 5.5-12(22) um.

Multiseptate conidia (Fig. 8F) occasionally forming in old cultures are brown with refractile septae, smooth walled, curved with a narrow base and truncate or pointed apex, 5-6um x 10-25um. Short hyaline filaments often remain attached at either end.

Colonies on PYE growing rapidly (70 mm in 14 days) are effuse, velvety, peach or salmon colored, becoming brown, reverse dark yellow, adhering poorly to the cellophane membrane and developing radial folds or detaching from the membrane at the periphery. No growth occurs at 37 C.

On cereal agar colonies just filling the petri dish by 14 days, are flat with small central umbo and few outward radiating folds, effuse, velvety, with dark grey aerial hyphae and yellow surface growth. Yellow exudate droplets form on the surface and the medium turns yellow or orange from diffusing pigment. On DSA the pigment is bright yellow.

There is no growth at 37 C.

Figure 8. A. Scytalidium acidophilum (UAMH 3460). B-F. Scytalidium flavo-brunneum (B,C-3487; D,F-617). G-I. Scytalidium state of Hendersonula toruloidea (3770). Fig. 8A. Chains of thick walled arthroconidia. Figs. 8B-8C. Fission arthroconidia forming on lateral branches. Figs. 8D-8E. Mature brown arthroconidia with hyaline apices. Fig. 8F. Multiseptate conidia. Figs. 8G-8I. Chains of arthroconidia forming on broad hyphae or narrower lateral branches. All x 830.

Figure 8. A. _Scytalidium_ _acidophilum_ [illegible]

[illegible]

[illegible]

8A. Chains of thick walled arthroconidia. Figs. [illegible] fission arthroconidia forming on lateral branches. [illegible] [illegible]-8E. Mature brown arthroconidia with hyaline a[illegible]. Fig. 8F. Multiseptate conidia. Figs. 8G-8I. Chains of [illegible] lateral branches. [illegible] x 830.


A
B
C
D
E
F
G
H
I

Habitat and Activities

Occurrence: Reported from soil, Georgia and Wyoming (Miller *et. al.*, 1957; Boeck, Hoehn, Westhead, Wolter, and Thomas, 1975).

Antibiotic Activity: Boeck *et. al.* (1975) reported antifungal activity of culture filtrates from an organism which they called *Geotrichum flavo-brunneum*. In assessing the antibiotic activity of the fungus, the authors observed that no other *Geotrichum* was known to produce antifungal agents. Because of its dematiaceous nature, *G. flavo-brunneum* has been transferred to *Scytalidium*, a genus having several other species which also produce antibiotics.

The antibiotic agent, isolated and characterized as an azasteroid (Michel, Hamill, Larsen and Williams, 1975) was found to have one major and six minor components. The major component was active against several pathogenic fungi, including *Candida albicans* and *Trichophyton mentagrophytes*, but minimally inhibitory to bacteria (Gordee and Butler, 1975).

Activity: *S. flavo-brunneum* digests cellophane after prolonged growth. Keratin is not attacked.

Material Examined

UAMH 617, received from Pfizer and Co., 1958, determined by B. Sloan; UAMH 3487, TYPE, from forest soil, Clarke Co.,

Georgia, by J.H. Miller, 1956, received from CMI as 100715.

*Scytalidium* state of *Hendersonula toruloidea* Nattrass 1933, Trans. Brit. mycol. Soc. 18:197

*History*

The arthroconidial state of *Hendersonula toruloidea* is frequently recovered from moribund tissues of fruit and trees in warm climates. Nattrass (1933) isolated the conidial state from die-back disease of stone fruit trees in Egypt and reported development of pycnidia only after prolonged growth on wood. He described the arthroconidial state as characteristic of *Torula*.

Wilson (1947) comparing a fungus isolated from branch wilt disease of Persian walnut with two others from damaged citrus trees in California, noted the similarity of his isolate to *H. toruloidea* but failed to find pycnidia. He described a new species, *Exosporina fawcetti*. Later Wilson (1949) induced pycnidial formation in wood experimentally inoculated with *E. fawcetti*, and concluded that *E. fawcetti* was the conidial state of *H. toruloidea*.

Oudemans (1904) created the genus *Exosporina* for a single species *E. laricis* which he found growing on twigs and needles of the larch (*Larix decidua*). Oudemans (1904) described conidia developing in chains from a stroma. Immature conidia at the base of the chain were cubical, whereas mature conidia at the apex became more rounded. The

description and illustrations of Oudemans (1904) suggest that *Exosporina* is an earlier name for *Ojibwaya* (Sutton, 1973) reported on stems of juniper.

The meristem arthroconidia of *E. laricis* differ from the fission arthroconidia of *H. toruloidea*. The *Scytalidium* state has been previously described but not named by Hughes (1952, 1953); therefore, a new combination is proposed in *Scytalidium*:

*Scytalidium fawcetti* (Wilson) Sigler comb. nov.

≡ *Exosporina fawcetti* Wilson 1947, Hilgardia 17(12):427, fig. 2 (Basionym)

Description

The *Scytalidium* state is characterized by dark grey-black effuse rapidly growing colonies often forming upright narrow threads of hyphae. Chains of arthroconidia develop on undifferentiated broad (4-7um) brown hyphae (Fig. 8H), often surrounded by a brown slime, or narrower (3-4um) lateral branches (Figs. 8G,8I). Arthroconidia (Fig. 8H) are 0-1 septate, smooth walled, brown, at first cylindrical truncate, rapidly rounding up and becoming barrel shaped or subglobose, 3.5-5um x 6.5-15um (2.5-7um x 2.5-10(15)um of Campbell, 1974). For a more detailed description, refer to Campbell (1974).

Habitat and Activities

Occurrence: Reported from fruit trees of many types

principally citrus and stone fruit (Nattrass, 1933; Wilson, 1947, 1949; Hughes, 1952; Calavan and Wallace, 1954) and Persian walnut, black walnut, European chestnut (Wilson, 1949; Calavan and Wallace, 1954). All are tropical varieties.

Pathogenicity: Recently, _H_. _toruloidea_ has been recovered from infections of the skin and nails in humans (Gentles and Evans, 1970; Campbell, Kurwa, Abdel-Aziz and Hodgson, 1973; Campbell, 1974). Most infections were reported in former natives of tropical countries now residing in Great Britain.

Pycnidia: Nattrass (1933) and Wilson (1949) induced pycnidial production in _H_. _toruloidea_ by growing the organism for long periods in wood.

Pycnidia have rarely been observed in isolates from human infections (Gentles and Evans, 1970; Campbell, 1974) although Campbell _et_. _al_. (1973) reported 5 of 8 isolates eventually developed pycnidia. Campbell (1974) induced pycnidial formation in 2 of 25 isolates by exposure to ultra-violet irradiation.

The single strain examined here (UAMH 3770) first formed pycnidia after exposure to ultra-violet light. During the process of drying, colonies (see Materials and Methods) are exposed overnight to a General Electric germicidal 15 w lamp, and when dried are placed in plastic

bags for storage. However, the agar was not sufficiently dried and the incompletely dried colony eventually formed pycnidia within the plastic bags. Subsequently, pycnidia developed on straw agar (chopped decomposing straw, 3%; agar 1.5%) after 49 days incubation at 25 C.

The pycnidial state has been described by Nattrass (1933), Wilson (1949) and Hughes (1952).

Activity: *H. toruloidea* digests cellophane. Campbell (1974) reported penetration of keratin of hair by single hyphae, and penetration of the nail.

Material Examined

UAMH 3770, from fingernail of Jamaican-born resident of England, at Univ. of Birmingham, by C.K. Campbell (M40)

## *Sporendonema* Desmazieres 1827

History

In 1827 Desmazieres described and illustrated *Sporendonema casei* which he found growing on outer crusts of cheese. In his original analysis, Desmazieres noted the affinity of *S. casei* to *Mucor crustaceus* Bulliard, *Aegerita crustacea* De Candolle, and *Oidium rubens* Link and suggested that none of these authors had correctly interpreted the manner of spore formation.

Desmazieres recognized two different forms of spore dissemination, one through release from the tip of the fertile hypha and the other by destruction of the outer hyphal wall. Disagreement among mycologists on the exact nature of spore formation and dehiscence has resulted in considerable confusion over the placement of this species.

S. casei was transferred to Torula Persoon as T. casei by Corda (1838) and later as T. sporendonema by Berkeley and Broome (1850). Berkeley and Broome disagreed with Desmazieres' interpretation of spore formation and noted "Corda's Torula Casei appears to be very different".

Bonorden (1851) followed Fries (1832) in accepting Desmazieres' Sporendonema casei as the correct name for this fungus, and later Bainier (1907) reviewed S. casei and described two new species.

However, Saccardo in 1882 referred Torula sporendonema Berk. and Broome to Oospora Wallroth, a nomen illegitimum, as O. crustacea (Bulliard) [=Mucor crustaceus Bulliard]. In 1886 Saccardo placed Sporendonema casei in synonymy with O. crustacea. Lindau (1907) accepted this transfer.

Sumstine (1913) further complicated the taxonomy of this fungus by stating that O. crustacea [=S. casei] was conspecific with Oospora lactis (Fres.) Saccardo = Geotrichum candidum Link. Sumstine also considered Chalara mycoderma Bonorden, another species referred to G. candidum,

as a possible synonym of _S. casei_.

Oudemans in 1886 according to Lindau (1907) emended _Sporendonema_ to include a new species _S. terrestre_. Although Lindau (1907) accepted this species, the status is uncertain. Arnaud (1952) in describing _Nyctalina lignicola_ noted its resemblance to _S. terrestre_.

In classifying a fungus isolated from curing blue cheese, Hammer and Gilman (1944) outlined the dilemma in naming the fungus. They reviewed the literature and recommended retention of the name _Sporendonema casei_ based on Desmazieres' original interpretation. In addition, the authors accurately and precisely recorded the conidium development of _S. casei_.

More recently, von Arx (1970) also accepted _Sporendonema casei_ Desmazieres.

## Generic Description

Hyphae septate, broad, hyaline; conidiophores lacking. The fertile hyphae are branched, often dichotomously, at first sparingly then more regularly septate, with septae forming first near the apex of the hypha. Arthroconidia are formed by condensation of the cytoplasm in adjacent or more often alternate segments separated by one or more empty cells. Maturing arthroconidia, retained within the original outer hyphal wall, become thick walled and are released by

fracture or lysis of the intervening empty cell. Arthroconidia are hyaline or pink or yellow, broad, mostly greater than 4um in diameter, smooth, thick walled.

*Sporendonema* is distinguished from *Coremiella* by its light colored hyphae and arthroconidia. Furthermore *Coremiella* may develop coremia especially on natural substrates, though rarely in culture. Coremium formation has not been reported in *Sporendonema*.

*Malbranchea* differs from *Sporendonema* in the width of the hyphae and arthroconidia which in *Malbranchea* rarely exceeds 4um.

Type Species

*Sporendonema casei* Desmazieres 1827, Ann. Sci. Nat. XI:247; Desmazieres 1827, Rec. Trav. Soc. Sci. Agric. Arts, Lille p. 185-187, pl. 3

= *Torula casei* (Desm.) Corda 1838, Icones Fungorum 4:8, fig. 36

?= *Mucor crustaceus* Bulliard 1782, Bull. Champ. Tab. 100

= *Oospora crustacea* (Bull.) Saccardo 1882, Michelia 2:545

?= *Aegerita crustacea* DeCandolle 1805, Fl. France 2:72

= *Torula sporendonema* Berkeley et Broome 1850, Ann. Mag. Nat. Hist. ser. 2, 5:460

?= *Oidium rubens* Link 1815, in Mag. Ges. Naturf. Freunde, Berlin 7:37

If Desmazieres' assertion that *Aegerita crustacea* DeCandolle is the same species as *S. casei* can be validated then this earlier specific epithet would take precedence. Similarly Link's brief description of *Oidium rubens* is difficult to interpret with certainty but it too may be an earlier specific epithet for *S. casei*.

## Description

*S. casei* is psychrophilic, growing and sporulating well at 8 C. Colonies grow slowly at 25 C with scant sporulation and fail to grow at 30 C. At 18 C, colonies on PYE agar reach a diameter of 30 mm and are flat, downy, creamy white front and reverse, whereas on cereal agar growth is more luxuriant, though slower, and the colony at first creamy white turns cinnamon yellow in color as sporulation commences. The reverse is pink and a pink pigment diffuses into the medium.

In microscopic preparations numerous large dark brown rod shaped crystals may be found.

Characteristically the fertile hyphae, arising as lateral branches from the vegetative mycelium, are slightly broader, mostly curved or loosely coiled, or straight (Figs. 9A,9B) and the cytoplasm is more dense. Septa, forming in more or less regular segments, are thick suggesting double septae (Fig. 9C). Concentration of cytoplasm occurs in alternate or adjacent segments (Fig. 9B)

and the internally developing arthroconidia synthesize new wall material. Maturing arthroconidia often become rounded at the corners and the original outer hyphal wall remains visible. Mature arthroconidia (Fig. 9D), released by fracture, sometimes remaining connected in groups of 2's or 3's , are yellow or hyaline, oblong, smooth (3)4-5um x 4-8(10)um. In old cultures, cinnamon colored arthroconidia are released not only by fracture of the hypha, but also by extrusion from the apex of the outer hyphal tube (Figs. 9E,9F) leaving a section of empty hypha, exactly as described by Desmazieres (1827).

## Habitat and Activities

Reported mostly from cheese; other records should probably be referred to *Scopulariopsis* according to Wakefield and Bisby (1941).

*S. casei* attacks neither cellophane nor keratin.

## Material Examined

UAMH 1506, from cheese, England by Galloway, 1949, received from CMI as 37084; UAMH 1508, from wooden cheese drum, London, England by Worthington, 1957, received from CMI as 68748; UAMH 3790, isolated at Hokkaido University, Japan by Sasaki (AHU 9107), 1963, from Tubaki, IFO, Japan as 7656.

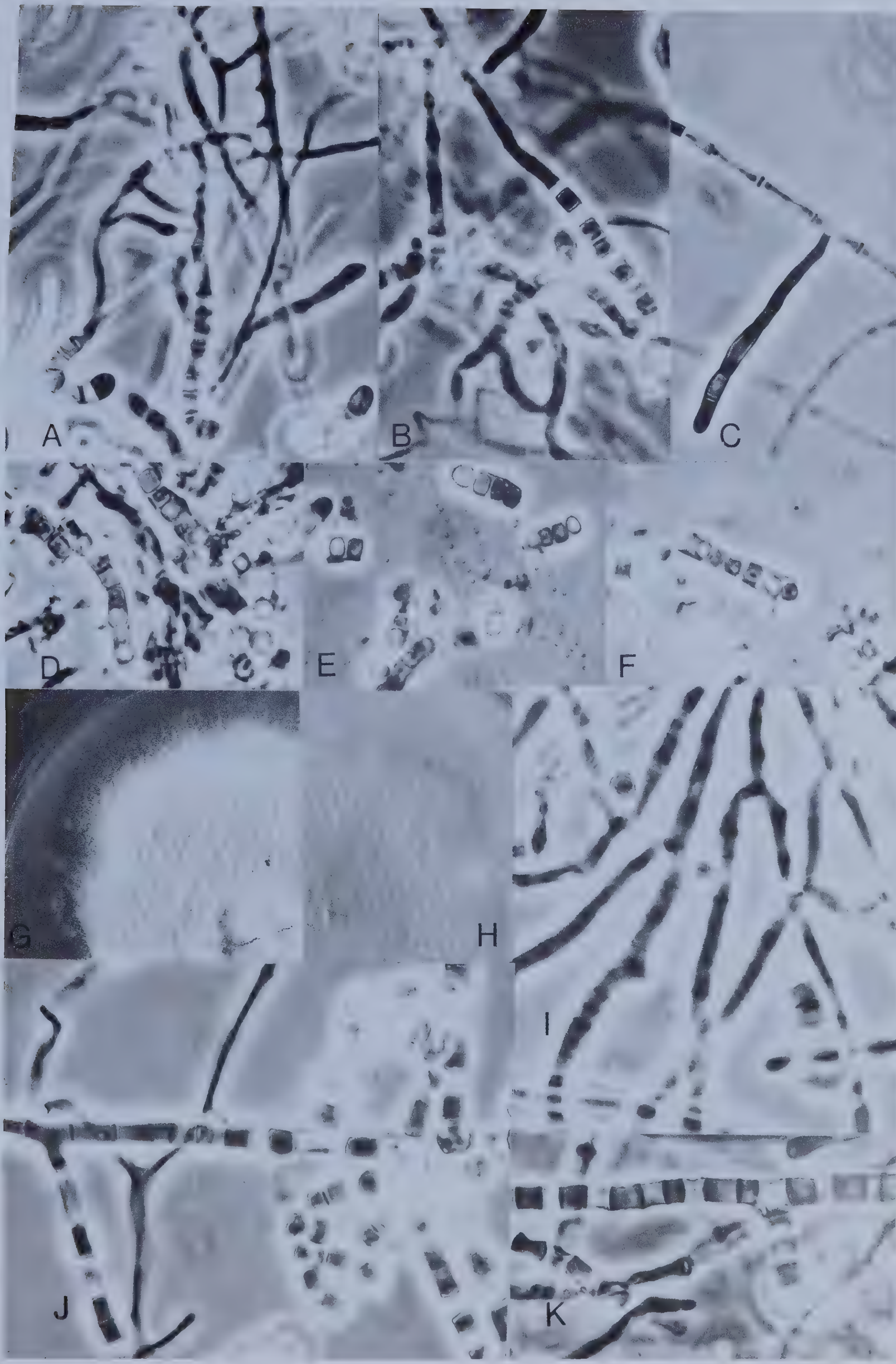
Figure 9. A-F. *Sporendonema casei* (A,B,D-F-UAMH 3790; C-1506). G-K. *Sporendonema purpurascens* (G-I,K-432; J-3791). Figs. 9A-9C. Alternate arthroconidia developing on straight or curved fertile hyphae. Fig. 9D. Mature arthroconidia. Figs. 9E-9F. Extrusion of arthroconidia from the apex of the outer hyphal wall. Figs. 9G-9H. Colonial morphology after 21 days at 25 C. G on PYE, H on cereal. Fig. 9I. Dichotomously branched fertile hyphae. Fig. 9J-9K. Alternate arthroconidia. Colonies x 1.0, others x 830.

[illegible]

Figs. 9A-9C. Alternate arthroconidia [illegible]

[illegible] arthroconidia. Figs. 9E-9F. Extrusion of arthrocon[illegible] from the apex of the outer hyphal wall. Figs. 9G-[illegible] Colonial morphology after 21 days at 25 C. G on PYE, [illegible]

[illegible] 9K. Alternate arthroconidia. [illegible]



Other Species

<u>Sporendonema purpurascens</u> (Bonorden) Mason et Hughes 1953 apud Wood 1957, Nature 179:328

= <u>Coprotrichum purpurascens</u> Bonorden 1851, Handbuch allgemeinen Mycologie p. 76, fig. 32 (Basionym)

= <u>Geotrichum purpurascens</u> (Bonorden) Saccardo 1886, Syll. Fung. 4:40

?= <u>Geotrichum roseum</u> Grove 1886, Syll. Fung. 4:40 fide Caretta 1959

= <u>Allonema roseum</u> Sydow 1934, Ann. Mycol. 32:283

?= <u>Sporendonema roseum</u> Grove var. <u>album</u> Arnaud 1952, Bull. Soc. Mycol. France 68:192, fig. 3,C,D

History

In 1851, Bonorden described <u>Coprotrichum</u> with two species, <u>C</u>. <u>purpurascens</u> and <u>C</u>. <u>cinereum</u>, illustrating both. Bonorden stated that the hyphae and spores of <u>C</u>. <u>purpurascens</u> were purple colored whereas those of <u>C</u>. <u>cinereum</u> were greyish colored.

In 1886, Saccardo made new combinations for each species in <u>Geotrichum</u>. Later, Windisch (1951) listed <u>C</u>. <u>cinereum</u> and <u>C</u>. <u>purpurascens</u> as synonyms of <u>Endomyces</u> <u>lactis</u> and subsequently Carmichael (1957) in emending <u>Geotrichum</u> included all three as synonyms of <u>G</u>. <u>candidum</u> Link.

At about the same time, Wood (1957) in a brief report on a disease of cultivated mushrooms caused by a so-called

'red *Geotrichum*' or 'lipstick mold' cited Mason and Hughes' identification of the causal agent as *Sporendonema purpurascens* (Bonorden) [≡ *Coprotrichum purpurascens*]. According to Wood, the fungus Bonorden described as *Coprotrichum purpurascens* was not identifiable with *G. candidum* and should be removed from *Geotrichum*. Certainly Bonorden's description of the hyphae and spores as purple colored would indicate a difference from *G. candidum*.

Although *Sporendonema purpurascens* is the name commonly applied to the fungus known as the 'lipstick mold' (Cole and Kendrick, 1969; von Arx, 1970; Kendrick, 1971, p. 164), Kendrick and Carmichael (1973) in a review of Hyphomycete genera list *Coprotrichum purpurascens* Bonorden as synonymous with *G. candidum*.

Caretta (1959) following Redaelli and Ciferri (1934) and Ciferri (1958) in accepting Oudemans' (1886, in Lindau 1907) emended description of *Sporendonema* Desmazieres, retained *Coprotrichum purpurascens* for the neotype culture of Wood (1957). However, Redaelli and Ciferri (1934) included in *Sporendonema* unrelated fungi (Dodge, 1935) and the fungus they called *Sporendonema epizoum* has been transferred to *Wallemia* as *W. sebi* (Fries) von Arx (1970) (see excluded species).

Caretta (1959) listed a number of synonyms for *C. purpurascens* including *Monilia miniata* Wallr. (?= *S. casei*, see Saccardo, 1886, p. 20), *Oospora crustacea* Sacc. [?= *S.*

*casei* Desm.], *Endoconidium luteolum* Delacr., *Oidium rubrum* Proks., *Scopulariopsis casei* Loubiere, ? *Oidium aurantiacum* Henneberg, *Coprotrichum crustaceum* Cif. et Red. and *Coprotrichum lutescens* Cif. et Red. I have not seen the original descriptions of these species.

## Description

Colonies on PYE (Fig. 9G) grow moderately rapidly (55-68 mm in 21 days) at first scant cottony white growth, then turning rose pink with a creamy white margin, dense, cottony or more often powdery, flat with a central plateau, dry, with fine surface cracks. The reverse is burnt orange in culture but burgundy colored when dried.

Growth on cereal agar (Fig. 9H) is slightly slower (45-60 mm in 21 days), less luxuriant, cottony, turning from white to peach or pale pink in color. The reverse is white and a tan or pinkish pigment diffuses into the medium below the colony.

There is no growth at 37C, but growth on oatmeal agar is faster at 30 C than at 18 C.

Alternate arthroconidia form on undifferentiated hyphae which may branch dichotomously (Fig. 9I). Arthroconidia (Figs. 9J,9K) are cylindrical or oblong, discoid in end view, hyaline at first, later pink, thick walled, smooth 4-7um x 4-12(15) um.

The formation of arthroconidia has been recorded by time-lapse photography (Cole and Kendrick, 1969).

## Habitat and Activities

*S. purpurascens* is most commonly associated with cultivated mushrooms.

This species is weakly cellulolytic requiring several weeks for digestion to occur. Keratin is not attacked.

*S. purpurascens*, one of several fungi tested by Komatsu (1969) inhibited *Pholiota nameko in vitro* at 25 C but failed at 12 C.

## Material Examined

From mushroom beds: UAMH 432 and 433, received from Kneebone, Pennsylvania State University, as isolates A and B respectively; UAMH 1497, NEOTYPE, Chesham, England, by F.C. Wood, 1951, from CMI as 45638; UAMH 3791, same strain as UAMH 1497, received from Tubaki, IFO as 7659 (CMI 45638).

## Species of Uncertain Status

*Sporendonema salicis* Bainier 1907, Bull. Soc. Mycol. France 23:24, pl. VI, fig. 7-9; Saccardo and Trotter 1913, Syll. Fung. 22:1240

*Sporendonema artemisiae* Bainier 1907, ibid, pl. VI,

fig. 10-12; Saccardo and Trotter 1913, Syll. Fung. 22:1240

Bainier reported this species on dead stems of the mugwort, _Artemisia_, forming well developed yellowish white or grey tufts (coremia?). It could be _Coremiella cubispora_ (Berk. et Curt.) Ellis. Bainier lists the size of the conidia as 1um12 - 1um25 and Saccardo and Trotter interpret these measurements as 1,12 u - 1,25 u. However, according to the illustrations (all at the same magnification), the diameter of the conidia of _Sporendonema artemisiae_ exceeds that of _S. salicis_ which was given by Bainier as 4um2. These measurements of Bainier appear to be in error.

_Sporendonema terrestre_ Oudemans 1886, Versl. en. Med. Konink. Ak. Wetensch. Amsterdam Afd. Natuurk. 3, ser. 2:115, pl. 1

_Sporendonema roseum_ v. _album_ Arnaud 1952, Bull. Soc. Mycol. France 68:192, fig. 3,C,D
?= _Sporendonema purpurascens_

_Excluded Species_

_Sporendonema sebi_ Fries 1849 = _Wallemia sebi_ (Fr.) von Arx 1970

_Sporendonema epizoum_ (Corda) Ciferri and Redaelli 1934 = _Wallemia sebi_

## PART II MALBRANCHEA SACCARDO 1882

### History

In 1882 Saccardo established the form-genus *Malbranchea* for a single species *M. pulchella* Saccardo and Penzig, found growing on damp cardboard in Rouen, France. Though providing no illustrations, Saccardo described the characteristic branched, arcuate, or curved fertile hyphae. However, in reporting the extrusion of conidia from the apex of the fertile hyphal branch, "ex apice ramulorum continuo exsilientibus", he failed to recognize the arthric nature of conidium dehiscence. Subsequently, Saccardo (1886) repeating his original description referred to the affinity of *Malbranchea* with *Oospora* Wallroth and *Glycophila* Mont. He provided no records of the cultural characteristics of the organism.

Since its original description, *Malbranchea* has been largely ignored by mycologists. Two other species were described in the period 1925-1935. Miehe (1907) described in detail, *Thermoideum sulfureum*, a thermophilic fungus, but Saccardo (1908) and Saccardo and Trotter (1913) considered it a synonym of *M. pulchella*. More recently, attention again focused on the thermophilic isolates when Cooney and Emerson (1964) reviewed the history of *Malbranchea* and

provided an excellent description. The history of the mesophilic and thermophilic species are given under *M. pulchella* and *M. sulfurea* respectively.

## Generic Description

Hyphae hyaline, septate; conidiophores absent. Primary hyphae straight, branched, mostly the same diameter, or slightly broader than the fertile hyphae, rarely exceeding 4um in diameter. Fertile hyphae, arising as branches from the primary hyphae, characteristically arcuate, in some species straight, branched, narrow 1.5-3um or wider, rarely exceeding 4um in diameter. Hyphae at first sparsely septate, then regularly so, with concentration of cytoplasm in alternate segments, separated by 1 or more empty cells; arthroconidia released by lysis or fracture of outer hyphal walls of intervening empty cells. Alternate arthroconidia smooth walled, cylindrical-truncate, curved or straight, hyaline, in age, yellow, tan, orange, greenish-yellow, in diameter not exceeding the width of the hypha which bears them.

## Type Species

*Malbranchea pulchella* Saccardo et Penzig 1882, Michelia 2:638

## Discussion

Although many other fungi form intercalary arthroconidia, *Malbranchea* species develop exclusively regular alternate arthroconidia. Species of *Malbranchea* can be divided into two groups, ones having arcuate or curved fertile hyphae and ones having more or less straight fertile hyphae. However, the primary hyphae of all species are straight, mostly slightly broader than the fertile branches, often forming intercalary arthroconidia which may predominate during early development. The size range of arthroconidia varies little among the species of *Malbranchea*. For this reason, the colonial morphology and color are important characters in distinguishing among the species. For color photographs of colonies, refer to the Appendix, Fig. 21.

## KEY TO THE SPECIES OF *Malbranchea*

Conidiophores absent .................................... 1

Conidiophores hyaline ...................... Try *Ovadendron*

Conidiophores pigmented ....................Try *Oidiodendron*

1. Fertile hyphae mostly greater than 4um in diameter ........................................Try *Sporendonema*
1. Fertile hyphae mostly narrower ......................... 2
2. Fertile hyphae arcuate or curved ....................... 3
2. Fertile hyphae straight, branched .....................11
3. Fertile hyphae tightly coiled, accessory conidia lacking ................................................... 4

3. Fertile hyphae curved, arcuate, accessory conidia present ............................................ 6

4. Colonies thermophilic; conidia mostly 2.5-4.5um broad ............................................M. *sulfurea*

4. Colonies not thermophilic; conidia mostly narrower ... 5

5. Colonies dark gold; perfect state *Myxotrichum* ............................................ M. *circinata*

5. Colonies tan, white or pale brown ......... M. *pulchella*

6. Colonies orange ........................................ 7

6. Colonies pale colored ................................. 9

7. Homothallic; perfect state *Auxarthron* .... A. *conjugatum*

7. Not homothallic; perfect state absent ................ 8

8. Arthroconidia intergrade with aleurioconidia formed laterally or terminally ............ M. *chrysosporoidea*

8. Aleurioconidia absent ................... M. *aurantiaca*

9. Colonies restricted, pinkish-buff; perfect state *Myxotrichum* ........................... M. *flavoroseus*

9. Colonies some other color ...........................10

10. Colonies ivory yellow, spreading, zonate; *Auxarthron* perfect state ........................... M. *albolutea*

10. Colonies buff or tan, rarely white, restricted or spreading .................................. M. *arcuata*

11. Branching of fertile hyphae regularly acute, arborescent ............................................ M. *dendritica*

11. Branching of fertile hyphae otherwise ...............12

12. Arthroconidia mostly narrow, 1.5-3um ................13

12. Arthroconidia mostly broader, 2.5-5um ...............17

13. Fertile hyphae, repeatedly branched, in dense tufts
.................................... *M. flocciformis*
13. Branching of fertile hyphae more restricted .........14
14. Colonies buff, tan or white ..........................15
14. Colonies lemon yellow ......................  *M. flava*
15. Colonies white, restricted .................  *M. gypsea*
15. Colonies buff or tan .............................16
16. Arthroconidia with refractile terminal walls, mostly 2-3um x 3.5-8(11)um ............................ *M. fulva*
16. Arthroconidia broader, 2.5-3.5um x 3.5-6um; uncinate appendages mostly present .................... *U. reesii*
17. Arthroconidia broad, 3-5um, closely spaced
............................ *M.* state of *C. immitis*
17. Arthroconidia narrower, 2.5-3.5um, irregularly spaced; uncinate appendages mostly present .......... *U. reesii*

*Malbranchea* resembles both *Coremiella* and *Sporendonema* in its conidium development. Whereas the former also differs in its dematiaceous nature, both are distinguished by the wide diameter of the hyphae and arthroconidia. Moreover, the perfect states of *Malbranchea* where known, are in the Gymnoascaceae. The perfect states of *Coremiella* and *Sporendonema* are largely unknown but Tubaki (1960) reported that *Onygena corvina* has a *Coremiella* conidial state.

Rebell and Taplin (1970) and Rippon (1974) noted the affinity of *Malbranchea* to some dermatophytes which may form

arthroconidia in culture. The relationship of the dermatophytes to perfect states in the Ascomycotina, family Gymnoascaceae, is well established (Nannizzia, Arthroderma). Malbranchea is related to the dermatophytes and Chrysosporium by its perfect states within the Gymnoascaceae, by its arthroconidia and its often keratinophilic nature. However, Malbranchea lacks the characteristic aleurioconidia of these genera.

## Perfect States

The conidial states of some Gymnoascaceae have been assigned to a number of form-genera including Chrysosporium (Carmichael, 1962; Apinis, 1970; Rebell and Taplin, 1970; Varsavsky and Orr, 1971; von Arx, 1971; Samson, 1972; Orr and Kuehn, 1972; Fennell, 1973), Oidiodendron (Orr and Kuehn, 1964a; Barron and Booth, 1966; Morrall, 1968; Muller and Pacha-Aue, 1968; von Arx, 1971; Fennell, 1973; Tokumasu, 1973), Geotrichum (von Arx, 1971) and Malbranchea (Kuehn, Orr and Ghosh, 1964; Hubalek, 1974a).

A closely related family, the Onygenaceae, is also known to have arthroconidial and aleurioconidial imperfect states (Tubaki, 1960; Malloch and Cain, 1971; Samson and van der Aa, 1973; Fennell, 1973). The lack of a phialoconidial state distinguishes the Gymnoascaceae from the Eurotiaceae (Stolk and Samson, 1972; Fennell, 1973).

Arthroconidial imperfect states have been described for

a number of species of Gymnoascaceae, referred to in earlier reports as oidia, or chlamydospores, or arthrospores (Kuehn, 1955b, 1959; Benjamin, 1956; Apinis, 1964). Orr *et al.* (1963b) defined the term arthroaleuriospore or arthroaleurie to differentiate alternate arthroconidia from the true *i.e.* fission arthroconidia of *Geotrichum*. However, Orr *et al.* (1963a,b) considered the arthroaleuries to be variable and therefore of little value in distinguishing among species.

Several *Malbranchea* species described in this report were found to have perfect states in *Myxotrichum*, *Auxarthron* and a new genus of Gymnoascaceae. In most cases the *Malbranchea* state is predominant and would be used for identification. Although beyond the scope of this work to study in detail all the arthroconidial states of the Gymnoascaceae, a few have been included in which the imperfect state may be confused with other species of *Malbranchea*. However, the cleistothecial state would be most often used for identification.

Species of *Auxarthron* have *Malbranchea* conidial states, two of which are described here. It is possible that the *Malbranchea* state may aid in differentiation among species of *Auxarthron* which are identified primarily by the morphology of the peridial hyphae and appendages (Orr *et al.*, 1963a; Samson, 1972). The ascospore morphology and size is quite uniform.

Cleistothecial production in the Gymnoascaceae is

stimulated by growth on oatmeal-salts agar (Weitzman and Silva-Hutner, 1967; Padhye *et al*., 1973) and by the use of several incubation temperatures such as 18 C, 25 C and 30 C. Strains failing to form cleistothecia at 25 C may do so readily at 18 C or 30 C, although the lower temperature more often stimulates development. In addition, keratinophilic isolates form cleistothecia when grown on dextrose-salts agar, a minimal nutrient medium, sprinkled with a keratinous substrate such as hair. Cleistothecial production is enhanced in cellulolytic fungi by growth on a cellophane membrane on the surface of the agar (see Materials and Methods) although the membrane tends to limit cleistothecial formation in keratinophilic isolates. Some, especially degenerate strains or ones maintained for long periods in culture, require an additional stimulus, usually prolonged growth, 6-10 weeks or more, or multiple transfers on oatmeal-salts agar.

## Ecology and Distribution

*Malbranchea* is a common soil fungus having a worldwide distribution. Species are mesophilic, thermotolerant or thermophilic and may be keratinolytic or cellulolytic.

Thermophilic isolates of the species *Malbranchea sulfurea* are cellulolytic and are most commonly associated with self-heating decomposing matter, or dung. Since its description by Cooney and Emerson (1964), this species has

been frequently reported (see *M. sulfurea*).

However, other records of *Malbranchea* are rare. Few reports of *Malbranchea* have occurred in surveys of keratinophilic fungi (Rebell and Taplin, 1970; Orr and Kuehn, 1972; Hubalek, 1974a; Hubalek and Balat, 1974; Rippon, 1974; Ajello and Padhye, 1974). However, *Malbranchea* is a little known genus, and isolates could have been listed as unidentified *Chrysosporium* species or more probably as imperfect states or as unidentified Gymnoascaceae (Emmons, 1954; Rees, 1967a,b; De Vroey, 1970; Hubalek, 1974a; Caretta and Piontelli, 1975). A number of species of *Malbranchea* have their perfect states in the Gymnoascaceae. Some keratinolytic thermotolerant species are imperfect states of *Auxarthron* whereas some mesophilic cellulolytic species are imperfect states of *Myxotrichum*.

With the exception of the *Malbranchea* state of *Coccidioides immitis* which is a human pathogen, there is little evidence to suggest that species of *Malbranchea* are pathogenic. However, arthroconidia of nonpathogenic species may be transient inhabitants of man and animals, which can be recovered from tissues of animals such as rodents and can survive passage through animals (Emmons, 1954; Kuehn *et al.*, 1964; Orr, 1968; Rippon, 1974). Some species of *Malbranchea* have been reported as fungi resembling *C. immitis* (Emmons, 1954, 1967; Plunkett, Walker and Huppert, 1963; Huppert, Sun and Bailey, 1967; Orr, 1968; Orr and Kuehn, 1972).

Temperature relationships and activity of isolates are important in differentiating species of *Malbranchea*. Incubation at 18 C or 30 C often stimulates formation of the perfect state. Since arthroconidium morphology in many species of *Malbranchea* is rather uniform, the colonial appearance becomes an important character. The morphology and growth rate of colonies at different temperatures provide considerable aid in differentiation among species.

a) *Malbranchea* species with arcuate fertile hyphae

*Malbranchea albolutea* Sigler et Carmichael sp. nov.

Perfect state: *Auxarthron* Orr, Kuehn et Plunkett 1963a

*Description*

Colonies on PYE (Fig. 10A) growing moderately rapidly (50-57 mm in 21 days) are pale ivory yellow, reverse yellow, powdery, dry and cracked. The colony is slightly raised with central umbo surrounded by two or three powdery zones and outer downy periphery. The periphery develops deep sectors which are flat, with scant aerial growth, identical to the margin. More downy colonies appear smoother with the zonate pattern and sectoring less evident.

On cereal, colonies (Fig. 10B) grow moderately rapidly (42-53 mm in 21 days) and are pale ivory yellow or creamy

buff, reverse yellow, powdery, dry, cracked by numerous tiny fissures, periphery downy, rarely sectoring. The surface is flat, with tiny central umbo or zone of scant aerial growth at center.

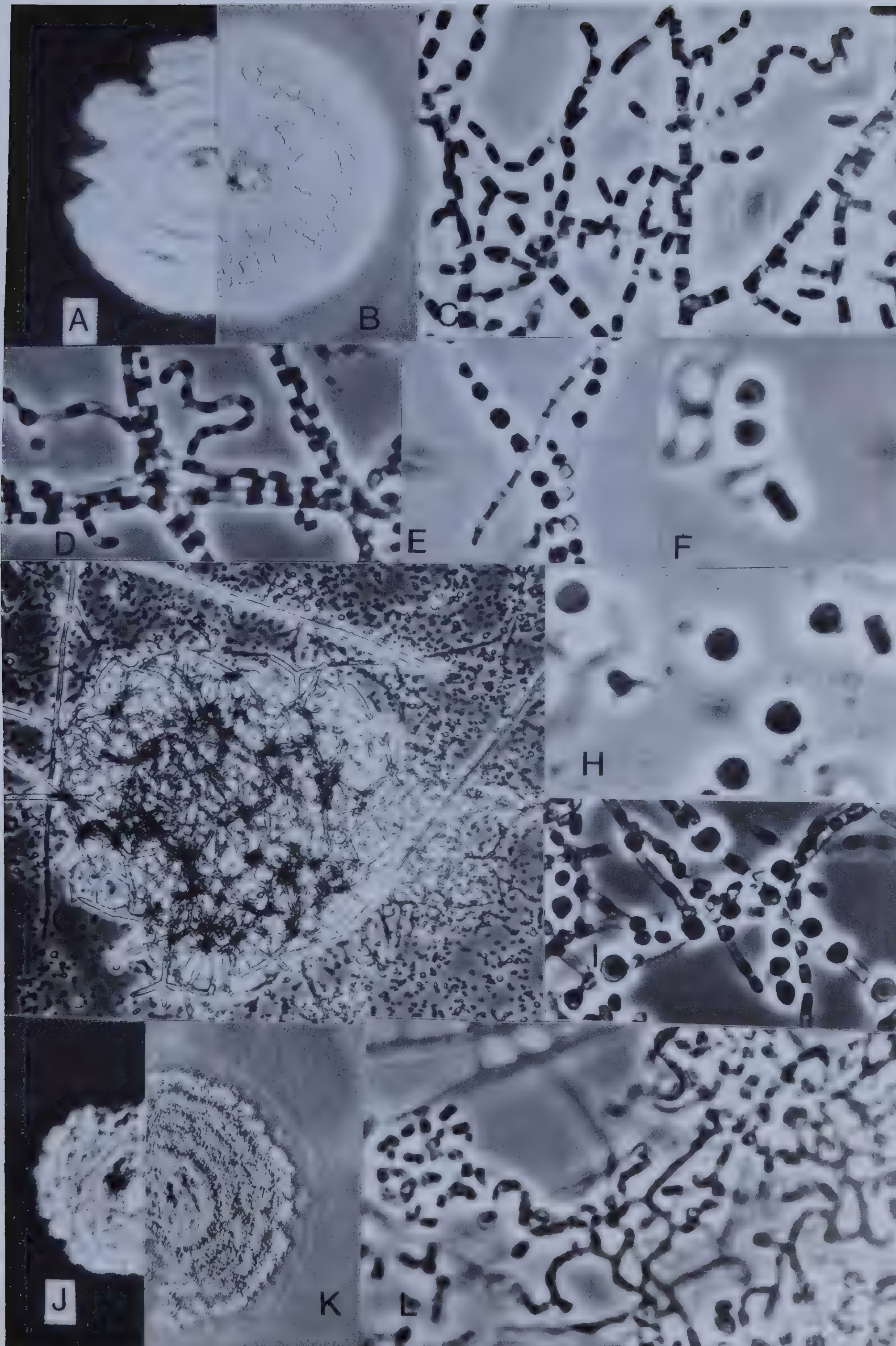
Most strains failed to grow at 37 C except UAMH 1846 which showed scant growth. The optimum temperature is 25-30 C.

Arthroconidia borne on narrow, arcuate or curved lateral branches (Figs. 10C,10D) are cylindrical sometimes curved, hyaline, in age yellow, 1.5-2um x (2.5)3-5(6)um. Arthroconidia formed by segmentation of the broader primary hyphae (Fig. 10D) are hyaline, cylindrical 2.5-3(4)um x (1.5)2-5(6.5)um. In age, racket hyphae, and enlarged, sub-globose, or irregularly shaped arthroconidia (Fig. 10E) are formed.

Cleistothecia (Fig. 10G) are spherical, discrete, 280-400um in diameter (excluding elongate appendages), brown, composed of a branched network of thick walled, delicately asperulate, septate, 3-5um in diameter, yellow-brown hyphae, with free apices terminating in bluntly pointed spines. Elongate appendages (Fig. 10G) arising from a bifurcate base are 400-800um in length, smooth, thick walled and yellow brown over half the length, tapering to a hyaline apex, straight, rarely uncinate. The hyaline portion is often broken off, leaving the tip blunt. Asci are evanescent, hyaline, 6.5-8um in diameter, 8-spored. Ascospores

Figure 10. A-H. Malbranchea albolutea (A,B,F,G-UAMH 2846; C-1846; D-2861; E-2848; H-3651). I-L. Malbranchea arcuata (I-1861; J-L-2519). Figs. 10A. Colonial morphology after 21 days at 25 C, x 1.0. A on PYE, B on cereal. Figs. 10C-10D. Alternate arthroconidia of arcuate branches and straight primary hyphae, x 830. Fig. 10E. Chains of enlarged arthroconidia, x 830. Fig. 10F. Globose ascospores, x 2300. Fig. 10G. Cleistothecium with elongate appendages, x 210. Fig. 10H. Finely asperulate ascospores, x 2300. Fig. 10I. Chains of enlarged arthroconidia, x 830. Figs. 10J-10K. Colonial morphology after 21 days at 25 C, x 1.0. J on PYE, K on cereal. Fig. 10L. Alternate arthroconidia borne on arcuate branches, x 830.

[illegible] (I-1861; J-L-2519). Figs. 10A. Colonial morphology [illegible] [illegible] straight primary hyphae, x 830. Fig. 10E. Chains of enlarged arthroconidia, x 830. Fig. 10F. Globose [illegible] ascospores, x 2300. Fig. 10I. Chains of enlarged arthroconidia, x 830. Figs. [illegible]-10K. Colonial morp[illegible] after 21 days at 25 C, x 1.0. J on PYE, K on cereal. Fig. [illegible] 830.


A
B
C
D
E
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4

(Figs. 10F,10H) are globose, delicately roughened, yellow (2.2)2.5-3.5um.

Holotype: soil, Utah, by G. F. Orr, 1967, UAMH 2846 (0-3508)

## Discussion

*Malbranchea albolutea* differs from other *Malbranchea* species having arcuate fertile hyphae such as *M. aurantiaca* and *M. arcuata* in its creamy white to pale ivory yellow colored colonies. Moreover, the perfect state is produced in culture if isolates are grown on a suitable medium.

Most strains of this species developed cleistothecia only after prolonged growth on oatmeal agar without cellophane. Even when cleistothecia are produced, the colony, on oatmeal agar, remains creamy white in color, never orange or brown. The cleistothecia are slow to mature requiring 6-8 weeks or longer before ascospores are produced. In some strains, even at maturity cleistothecia are formed which appear relatively empty, containing no ascospores. However, ascospores can be induced by repeated transfers on oatmeal agar and growth at 25 C or 30 C. The ascospores appear smooth at low magnification and the rough surface is often difficult to detect even at high magnification (x2700)(Figs. 10F,10H).

The perfect state of *M. albolutea* is assigned to *Auxarthron*, a genus distinguished from other genera of the Gymnoascaceae by its net-like anastomosed peridial hyphae

bearing prominent enlarged septae, described as 'knuckle-joints' and by its ascospore morphology (Orr _et_ _al_., 1963a). These generic characters described for _Auxarthron_ have not been accepted by some others. Apinis (1964) noted that enlarged septae also occurred in some species of _Gymnoascus_ and in _Myxotrichum_ _herbariense_ [= _Tripedotrichum_ _herbariensis_ Orr and Kuehn, 1964b]. Therefore, he retained _Auxarthron_ and _Pseudogymnoascus_ Raillo as subgenera of _Gymnoascus_ based on a broader concept of the genus _Gymnoascus_. Apinis included in the subgenus _Auxarthron_ species having uncinate or straight, elongate peridial appendages. Udagawa (1966) followed Apinis in retaining _Gymnoascus_.

Historically, the character of the peridial hyphae and appendages has received emphasis in classification of genera of the Gymnoascaceae. However, Samson (1972) in a study of _Pseudogymnoascus_, _Gymnoascus_ and _Auxarthron_ pointed to the variability of these characters during different stages and cultural conditions of growth, and suggested that more reliable characters for differentiation were morphology of the ascomatal initials, asci and ascospores. Samson (1972) retained all three genera and distinguished _Auxarthron_ from _Gymnoascus_ and _Pseudogymnoascus_ on the basis of its globose or subglobose, roughened or echinulate ascospores and arthroconidial or aleurioconidial imperfect state.

Eight species have been described in _Auxarthron_ (Orr _et_

*al*., 1963a; Orr and Kuehn, 1971, 1972). Samson (1972) suggested that a review of the number of species of *Auxarthron* should be considered. Species of *Auxarthron*, which have a rather uniform ascospore size and morphology are difficult to differentiate by the morphology of the peridial hyphae and their appendages.

The perfect state of *M*. *albolutea* most closely resembles *Auxarthron* *thaxteri* (Kuehn) Orr and Kuehn [= *Myxotrichum* *thaxteri* Kuehn, 1955b]. Orr *et* *al*. (1963a) described *Auxarthron* *brunneum* based on Rostrup's description of *Myxotrichum* *brunneum* and placed *M*. *thaxteri* Kuehn in synonymy. Apinis (1964) compared Rostrup's type specimen of *M*. *brunneum* with the type material of *Gymnoascus* *umbrinus* Boudier and found the two identical. Apinis placed *M*. *brunneum* in synonymy with *G*. *umbrinus* v. *umbrinus* and retained Kuehn's *M*. *thaxteri* as a variety, *G*. *umbrinus* var *thaxteri*. Based on their evaluation of the type materials of Rostrup and Boudier, Orr and Kuehn (1971) disagreed with Apinis' (1964) evaluation of *M*. *thaxteri* and they proposed to retain it as a separate species, *Auxarthron* *thaxteri*. *Myxotrichum* *brunneum* Rostrup was placed in synonymy with *A*. *umbrinum* (Boudier) Orr and Plunkett, but *Auxarthron* *brunneum* sensu Orr and Kuehn (1963a) was placed in synonymy with *A*. *thaxteri* since the description of this species was based on the type culture of *M*. *thaxteri*.

The perfect state of *M*. *albolutea* appears to fit the

description of Orr *et al.* (1963a) of *A. thaxteri* [= *A. brunneum*]. Indeed, the perfect state of one of the strains examined by them (O-1024, UAMH 1117) is identical with the perfect state described here. However, an examination of the type strain of *A. thaxteri* (UAMH 3912, NRRL 1714, ATCC 15598, O-532) and the description of Kuehn (1955b) revealed a number of differences from *M. albolutea*. First, the ascospores of the latter are globose, delicately roughened, (2.2)2.5-3.5um in diameter, compared to the ascospores of *A. thaxteri* which are ovoid, delicately roughened, 2-2.2um x 2.8-3.2um (2.6-2.7um x 2.7-2.8um according to Kuehn, 1955b); the cleistothecia of both are similar in size and shape but the elongate appendages of *A. thaxteri* are shorter, 300-400um in length, and mostly uncinate; the arthroconidial state, *M. albolutea*, is prominent in early stages of growth, permitting recognition of this species, whereas the *Malbranchea* state of *A. thaxteri* is associated with the appearance of ascomatal initials in culture.

At present, the primary reason for not including the perfect state of *M. albolutea* in *A. thaxteri* is the difference in size and shape of the ascospores. Obviously, this difference in size is small and would normally be part of the range of variation for this species, but the shape of ascospores within a species should be uniform. Indeed, the ascospores of all isolates of *M. albolutea* are uniformly globose. The ascospores of *Auxarthron* species are not only globose or subglobose as suggested by Samson (1972) but also

oblate and may be delicately roughened to spiny or echinulate-reticulate. For the present, the perfect state of _M_. _albolutea_ has not been assigned to _A_. _thaxteri_ nor has it been described as a new species, because the species of _Auxarthron_ should be reevaluated before additional ones are added.

## Habitat and Activities

Recovered from soil or dung, in the USA, from Utah, Colorado, California and Wyoming and in Hungary.

_M_. _albolutea_ is keratinolytic, producing moderate digestion of hairs without the aid of penetrating bodies. Cellophane is not attacked.

## Material Examined

From soil: UAMH 1117, Oildale, California, from Orr as 0-1024; UAMH 2632, soil?, Cache la Poudre River, Colorado, by W.B. Cooke, 1965, from G. Barron, Ontario Agricultural College as 10527; UAMH 2846, TYPE, Utah, by G.F. Orr, 1967, as 0-3508; UAMH 2848, fabric bait technique, Hungary, by G.F. Orr, 1967, as 0-3515; UAMH 2861, Utah, by G.F. Orr, 1967, as 0-3509; UAMH 3651, Wendover, Utah, from Orr as PO-0062; UAMH 3911, grasslands, Laramie, Wyoming, by M. Christensen (TC-22), from Orr as 0-1030;

From dung: UAMH 1846, rat, Mercur, Utah, from Orr as 'Merc' (?0-1089);

Material of <u>Auxarthron thaxteri</u>: UAMH 3912, TYPE, dung of opposum shrew (<u>Selenodon</u>), Haiti, by R. Thaxter, from Orr as 0-532 (NRRL 1714, ATCC 15598)

<u>Malbranchea arcuata</u> Sigler et Carmichael sp. nov.

<u>Description</u>

Colonies on PYE (Fig. 10J) growing moderately slowly (13-30 mm at 14 days) are slightly raised with small central umbo or few large smooth folds across the surface. The center is tan, powdery, dry and cracked and the margin downy, creamy white and flatter, reverse yellow or yellowish tan. Colonies of some strains, growing more rapidly (45 mm in 14 days) are flatter, downy, creamy-white with pale tan center, reverse yellow, smooth or with few outward radiating folds. Growth at 37 C is variable. Of the five strains tested, four (UAMH 1861, 2519, 2570 and 3910) failed to grow, but the other (UAMH 2983) showed scant growth.

On cereal, colonies (Fig. 10K) grow moderately rapidly (23-40 mm in 14 days) and are tan, reverse buff, flat, powdery, in some strains appearing slightly zonate, in others, patchy. Scant brown pigment appears below the colony by 21 days. This brown pigment is also produced when strains are grown on oatmeal agar.

Arthroconidia borne on narrow arcuate or curved lateral branches (Fig. 10L), are cylindrical, often curved, smooth,

hyaline at first, later tan, (1.5) 2-3um x 3-6um. Arthroconidia, formed by segmentation of the straight primary hyphae are hyaline, cylindrical, slightly broader, mostly 3um in diameter, and predominate in young cultures. In age, some arthroconidia become enlarged, subglobose or irregularly shaped (Fig. 10I).

No other spore state was observed.

Holotype: soil, Dugway, Utah, UAMH 1861 (0-1094, DPG 105)

## Discussion

The tan powdery colonies and arthroconidia borne on arcuate hyphae are characteristic of *M. arcuata*. Some variation in colonial morphology and growth rates is evident among strains included in this species. However, there appears to be little justification for removing any to separate species since the microscopic morphology is uniform.

*M. arcuata* is distinguished from other *Malbranchea* species having buff or tan colored colonies, such as the *Malbranchea* state of *Uncinocarpus reesii* and *Malbranchea fulva*, by its arcuate hyphae. *M. aurantiaca* and *M. albolutea* differ in having orange colored and pale ivory yellow colored colonies respectively.

## Habitat and Activities

Recorded from soil, dung of dog and birds, hair of rodents, USA and Yugoslavia (Hubalek, 1974a,b).

*M. arcuata* produced slight to moderate digestion of hairs without the aid of penetrating bodies. Cellophane is not attacked.

Material Examined

From soil: UAMH 1861, TYPE, Dugway, Utah, from Orr as DPG 105 (0-1094); UAMH 2519, Austin, Texas, by Alexopoulas, 1965, from Orr as Alex(50); UAMH 3877, ?dog dung, by Alexopoulas (50), from Orr as 0-3263; ?UAMH 2570, mixed with plant debris, Columbia, South America, by A. Rostrepo, from C.W. Emmons, Dept. Health, Bethesda, Maryland, as B 27-40; From pellets of *Merops apiaster* in nest, Pesirevo, Yugoslavia: UAMH 3844, by Hubalek, 1968, as 232B; ?UAMH 3845, by Hubalek, 1968, as 236A;
From hair of small rodents: UAMH 3847, Vojnik, Yugoslavia, by Hubalek, 1968 as JU 1623/2; UAMH 3902, *Apodemos* sp., Yugoslavia, by Hubalek (BH 14), from Orr as 0-3411; From feathers: UAMH 3842, *Corvus monedula*, Furka, Yugoslavia, by Hubalek 1968, as 153A; From unknown sources: UAMH 2983, from R.S. Pore, West Virginia Univ. Medical Center, Morgantown as 704; UAMH 3910, from J. Ellis, NRRL 6089.

*Malbranchea aurantiaca* Sigler et Carmichael sp. nov.

Description

Colonies on PYE grow moderately rapidly (64-70 mm in 21 days) extending in spiral zones (Fig. 11A). The central zone is umbonate, dark yellow-gold or orange brown in color, velvety or powdery; succeeding zones vary from dark gold or yellow-orange to creamy white and floccose at the periphery, reverse dark orange. Continuing in a spiral pattern, the margin emerges beneath the outermost zone and gradually extends around the colony, at first white with scant aerial growth eventually creamy white, floccose and dense. Sometimes the zonate pattern is not as distinct but the characteristic color remains.

At 37 C growth on PYE (Fig. 11C) is almost as rapid (44-62 mm, rarely up to 72 mm in 21 days) flat with irregular folds, pale orange or tan, dark orange reverse, downy. Droplets of yellow or brown exudate occasionally form on the surface.

On cereal, colonies (Fig. 11B) grow slightly slower (53-62 mm in 21 days). In early stages, colonies are dome shaped, floccose and white and as sporulation commences, the center becomes flattened, dark yellow-gold, orange-brown or tan, powdery or granular, sharply demarcated from a peripheral floccose creamy white raised outer ridge (Fig. 11B). Two gradations can occur around the center with an inner region, pale tan or creamy white and floccose, slightly raised above the flat center. The reverse is yellow. A yellow pigment diffuses into the agar in some

strains.

At 37 C, the colony (Fig. 11D) is characteristically slower growing (42-56 mm in 21 days) flat, dark tan or brown, powdery or granular, dense or patchy, with orange surface growth forming a distinct orange glabrous margin 5-8 mm wide. The reverse is light or dark orange.

There is no growth at 45 C, the optimum range being 25-30 C.

Arthroconidia borne on narrow arcuate or curved lateral branches (Figs. 11E,11F) are cylindrical, often curved (Fig. 11H) hyaline at first, later orange or tan in mass, smooth 1.5-2um x (2)3-5.5(6)um. Arthroconidia formed on the straight primary hyphae (Fig. 11G) are hyaline, broader, cylindrical 3-4um x (2)3-7(7.5)um and predominate in young cultures. The development of racket hyphae is common in the primary hyphae with swelling at the septum up to 9um in diameter. Rarely, intercalary chlamydospores are seen.

a) Atypical strains (UAMH 1569, 1853, 1709, 2844)

Four strains differing in several characters have not been summarized in the description above. However, all four are indistinguishable microscopically (Figs. 11I,11J) from *M. aurantiaca* and they are retained here until similar isolates are found which would justify their removal to a separate species.

Figure 11. <u>Malbranchea aurantiaca</u> (A,B-UAMH 3704; C,D,H-1707; E,G-3599; F-3705; I,J-2844; K-1707x3599). Figs. 11A-11D. Colonial morphology after 21 days. A and C on PYE, B and D on cereal; C,D at 37 C, others at 25 C. Figs. 11E-11J. Alternate arthroconidia of curved branches and straight primary hyphae. Fig. 11K. Zone of inhibition in cross of 1707x3599 inoculated in separate parallel streaks. Colonies x 1.0, others x 830.

[illegible] [illegible] aurantiaca (A,B-[illegible]

[illegible] [illegible] [illegible]2795; I,J-2844; K-1707[illegible]

[illegible] [illegible] [illegible] after 21 days. [illegible]

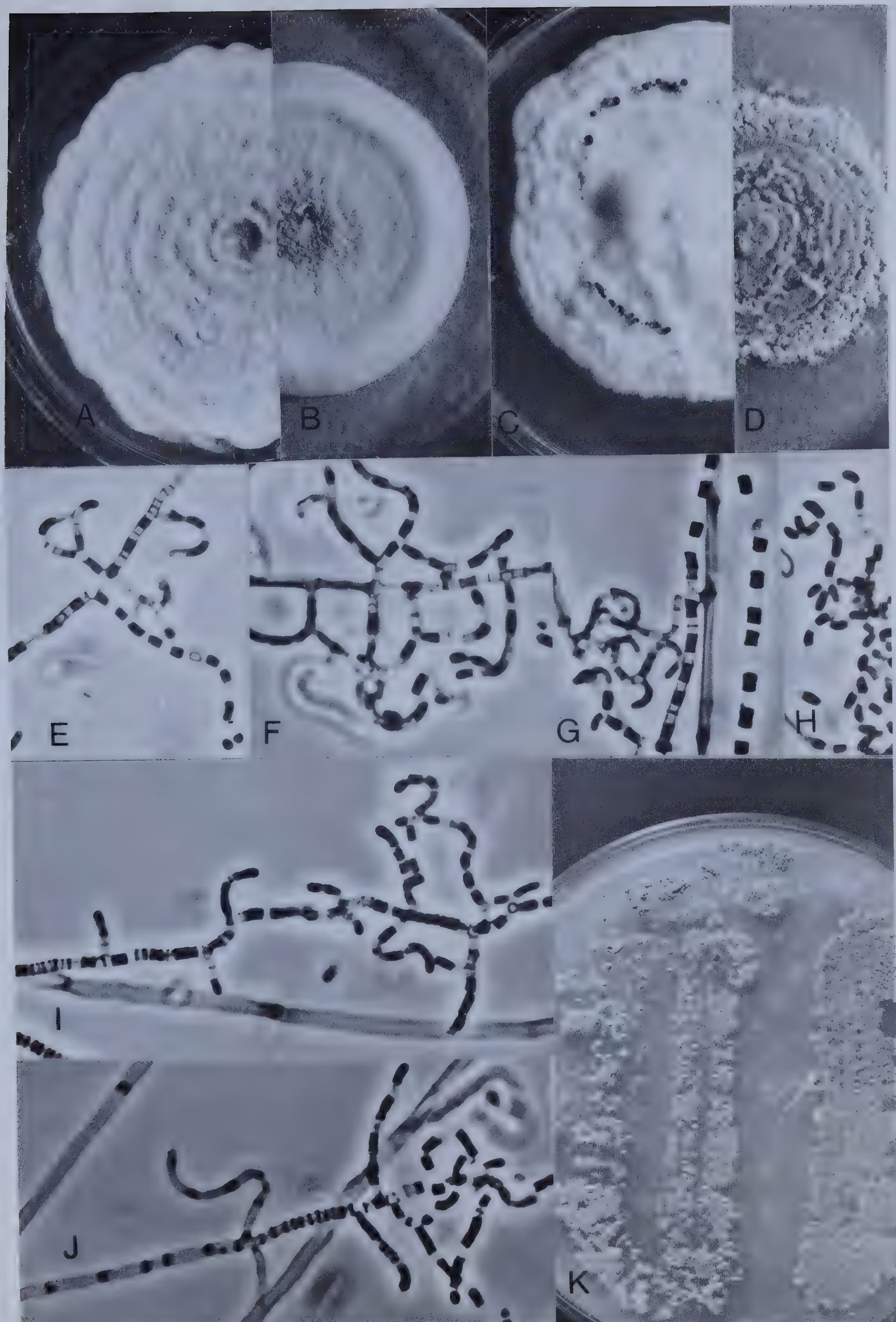
[illegible] [illegible]; C,D at 37 C, others at [illegible]

[illegible] [illegible] [illegible]conidia of curved [illegible]

[illegible] [illegible] [illegible]. Fig. 11K. [illegible] [illegible]

[illegible] [illegible] [illegible]ted in separate [illegible] [illegible]

[illegible] [illegible] [illegible] x 530.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Two strains (UAMH 1569 and 1853) are identical to each other. These strains differ from typical strains in having paler yellow orange colored colonies and in lacking the distinct spiral zonate pattern. At 37 C growth is more restricted (22-33 mm on PYE and 7-14 mm on cereal in 21 days.

UAMH 2844 is similar to strains UAMH 1569 and 1853 in its yellow orange colored colonies. On PYE the colony is smooth, not zonate, domed with a central umbo and on cereal, it is flat with sparse aerial growth at the outer periphery and a raised more dense central zone. This strain differs from all others in growing more robustly and rapidly at 37 C (75 mm on PYE and 63 mm on cereal in 21 days).

Strain 1709 differs from the others in having slower growing (46 mm on PYE and 42 mm on cereal after 21 days), dark orange colonies, velvety or downy. On cereal, the colony is zonate with a central umbo and on PYE it is flat with broad white margin and central orange umbo. There is no growth at 37 C after 21 days. The arthroconidia are slighter broader, 2-3um in diameter. Late in the study we received another isolate (UAMH 3878) which greatly resembled UAMH 1709. These two strains may eventually require transfer to a separate species, but it seems preferable to retain them here until a larger number of isolates has been studied.

Holotype: lab plate contaminant, Dugway, Utah, UAMH 3599 (O-

1526)

## Discussion

In its orange colored colonies, *M. aurantiaca* resembles the *M.* state of *Auxarthron conjugatum*. However, *A. conjugatum* is homothallic and regularly forms the perfect state in culture. Furthermore, the *M.* state of *A. conjugatum* does not develop the characteristic spiral zonate pattern on PYE, and growth at 37 C is much more restricted.

*M. chrysosporoidea* also forms orange colored colonies but lacks arcuate fertile hyphae. The color of the colony distinguishes *M. aurantiaca* from *M. arcuata* which forms buff colored colonies and *M. albolutea* which forms pale yellow or creamy white colonies.

## Habitat and Activities

Occurrence: Isolated from soil or dung, India, Central America, Australia, USA and Belgium.

Mating tests: Crosses were performed in duplicate by each of the mixed suspension (method B) and parallel streak plate (method C) procedures (see Materials and Methods) with the addition of autoclaved human hair.

With a single exception, no cleistothecia were seen in any cross after 42 days. In the mixed suspension cross of UAMH 1707 x 3705 a single cleistothecium of the '*Auxarthron*'

type (Orr, 1963a) bearing elongate uncinate appendages and few spiny oblate ascospores was seen. After a further two weeks growth no other cleistothecia were found nor were any discovered on the streak plate. The incompatibility of many of the strains was evident by a narrow or wide zone of inhibition on the streak plates (Fig. 11K, Table III).

Failure to obtain the perfect state in this species was surprising considering the close resemblance to some species of *Auxarthron* especially *A. conjugatum*. Indeed, several single ascospore isolates were attempted from a number of strains of *A. conjugatum* in the belief that *M. aurantiaca* might be the imperfect state of this species. However, all single ascospore isolates proved to be homothallic (see *Auxarthron conjugatum*).

The occurrence of a single fertile cleistothecium suggests either latent homothallism in one of the two strains tested or heterothallic incompatibility of the type frequently observed in some Gymnoascaceae. Incompatibility results in infertile crosses between F1 progeny and parent strains and between wild-type isolates crossed with each other or with tester strains of the opposite mating type. In the latter case, wild type isolates may only cross with the more fertile progeny of the parent strains (Kwon-Chung, 1971, 1972; Padhye and Carmichael, 1971, 1973).

Activity: *M. aurantiaca* produces moderate to marked

TABLE III

INCOMPATIBILITY AMONG CROSSES OF MALBRANCHEA AURANTIACA

| STRAIN | 1707 | 1709 | 1778 | 1853 | 2844 | 3524 | 3599 | 3660 | 3704 | 3705 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1569 | I | I | - | NI | - | - | NI | NI | - | - |
| 1707 | | I | - | I | - | NI | I | NI | - | + |
| 1709 | | | NI | - | - | - | NI | I | - | - |
| 1778 | | | | - | - | - | - | - | - | - |
| 1853 | | | | | - | - | NI | - | - | NI |
| 2844 | | | | | | S | - | - | - | - |
| 3524 | | | | | | | NI | - | S | NI |
| 3599 | | | | | | | | I | S | NI |
| 3660 | | | | | | | | | I | NI |
| 3704 | | | | | | | | | | NI |

Legend:
- \- No cleistothecia formed on duplicate crosses
- \+ Single fertile cleistothecium formed on mixed suspension plate
- NI Narrow zone of inhibition (<3 mm) and no cleistothecia
- I Moderate zone of inhibition (>3 mm) and no cleistothecia
- S Zone of stimulation at interface of mating strains

digestion of hairs but fails to attack cellophane.

## Material Examined

From soil: UAMH 1569, chicken feeding ground, Tonasi, Panama, collected H.P. Puri, isolated by G.F. Orr, as O-2505; UAMH 1705, garden, Tonasi, Panama, by G.F. Orr, O-2535; UAMH 1707, chicken feeding ground, Tonasi, Panama, by G.F. Orr, O-2531; UAMH 1709, South California, by G.F. Orr, O-584; UAMH 1710, pig yard, Tonasi, Panama, by G.F. Orr, O-2534; UAMH 1778, from mixed culture received as 288c (soil, Guatemala) from Orr; UAMH 1853, Australia by Warcup, from Orr as O-3598 (A 270/1); UAMH 2844, Belgium, by De Vroey, 1966, from Orr as O-3178; UAMH 3660, from Ellice Island, Alaska, from Orr as O-3035; UAMH 3818, Italy, by Varsavsky (I36) from Orr as O-3437; UAMH 3878, Inogmar area, California, from Orr as O-683; O-3482, Somalia, by DeVroey (RV 20443) from Orr; O-2592, Panama, from Orr; From dung: UAMH 3483, mouse, India, from Orr as O-3150; UAMH 3524, rat, India, from Orr as O-3733 (NRRL A-19283); UAMH 3704, rat, India, from Orr as O-1163; UAMH 3053, lizard, Chihauhua, Mexico, by R.K. Benjamin, 1964, RSABG, as 1468; From other sources: UAMH 3599, TYPE, plate contaminant, Dugway, Utah, by G.F. Orr as O-1526; UAMH 3705, plate contaminant, Dugway, Utah, by G.F. Orr as O-3214; UAMH 3879, hair from ringworm lesion on horse, Riverton, Utah, from Orr as O-3710

*Malbranchea chrysosporoidea* Sigler et Carmichael sp. nov.

Description

Colonies on PYE (Fig. 12A) growing rapidly (66-83 mm in 21 days), are characteristically bright tangerine orange, reverse orange. Nonsporulating floccose areas at the center or periphery are white or pale yellow. Colonies are dense, powdery or velvety, slightly raised, with a central domed downy or floccose umbo, scarcely zonate, demarcated by 2 or 3 changes in color from dark orange at the center to a light or white periphery, or distinctly zonate with 6 or 7 concentric zones. The margin, crenate or entire, develops scant aerial hyphae closely adpressed to the cellophane, then becomes floccose, white or pale orange. No pigment is apparent but most strains formed yellow, orange or white exudate droplets.

At 37 C, growth on PYE is more restricted (Fig. 12C) (25-31 mm in 21 days) adhering poorly to the cellophane, with the center lifting up, becoming convoluted or folded, with new growth appearing below. The center turns dark tan, brown, or tawny, powdery and with new growth, downy, orange. If the colony remains firmly attached to the cellophane, the diameter reaches to 44 mm and the colony is orange buff in color.

Colonies on cereal (Fig. 12B) growing slightly more slowly (61-68 mm in 21 days) are flat, mostly with a small central umbo, powdery or granular, patchy or dense, often

sectoring, scarcely zonate with changes in color from dark orange or tawny to yellow at the periphery, reverse orange.

At 37 C, growth is slower (18-36 mm in 21 days) orange or buff, rarely white, granular or downy, flat with a brown pigment appearing below the colony (Fig. 12D).

Fertile hyphae bearing arthroconidia are of two types. Straight, slightly broader, branched primary hyphae, 2-4um in diameter, which first produce intercalary arthroconidia (Figs. 12E,12F) and later form multiple straight or curved, deflexed lateral branches (Fig 12E,12I) bearing intercalary arthroconidia intergrading with aleurioconidia formed terminally or directly on the sides of the hypha. The width of the arthroconidia and aleurioconidia forming on the same hypha are identical with the diameter of the hypha. Intercalary arthroconidia are cylindrical-truncate, hyaline, later pale orange, orange in mass, (1.5) 2-4um x (2) 3-9(13) um. Aleurioconidia are smooth, hyaline, later pale orange, orange in mass, cuneiform 2-3um x (2.5) 3-5.5 (6.5) um.

No other spore state was observed.

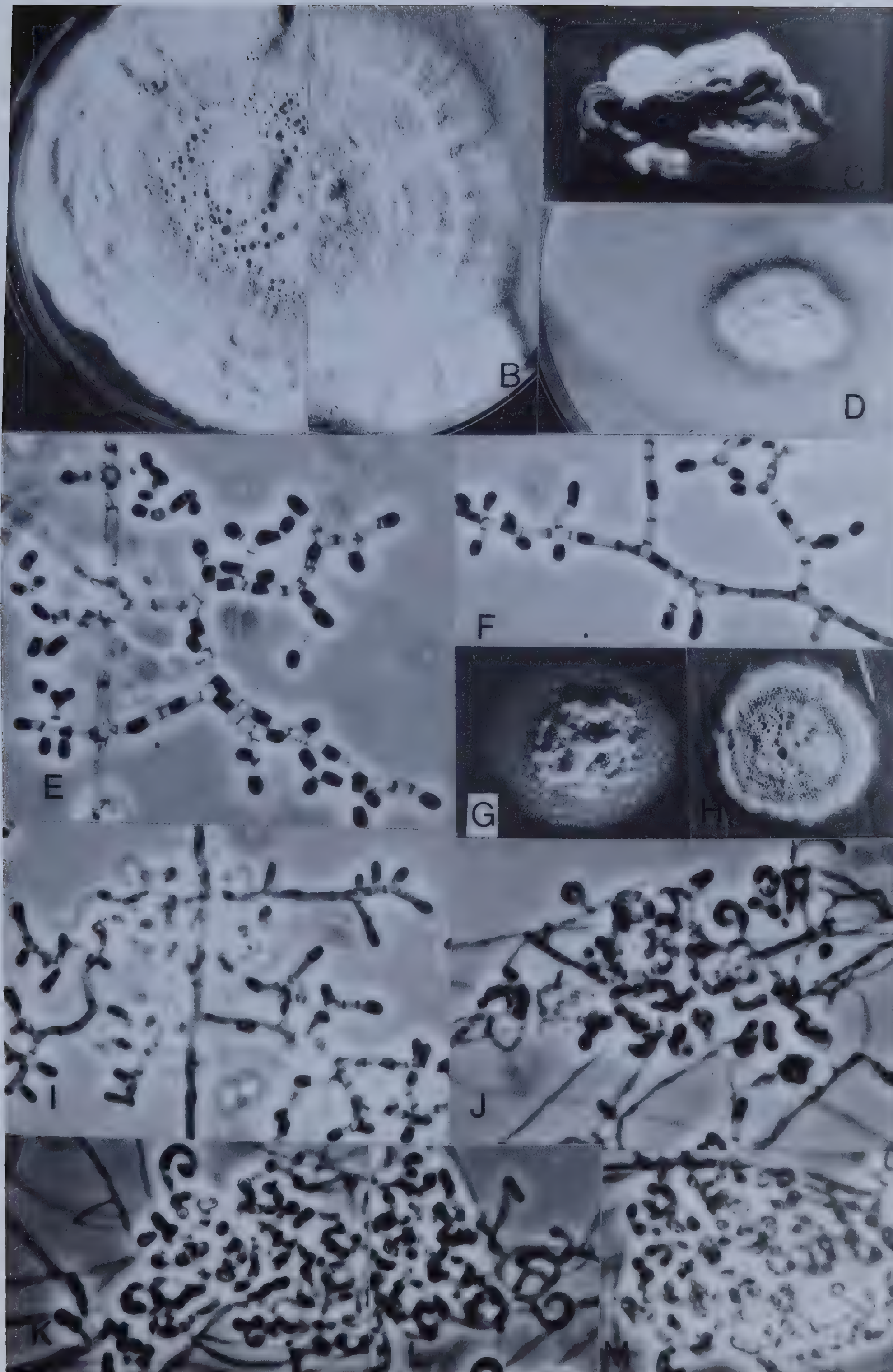
Holotype: soil, Arizona by C.W. Emmons (E5003), UAMH 1032 (O-1525)

<u>Discussion</u>

This species is a form intermediate between <u>Malbranchea</u> and <u>Chrysosporium</u>. Though formation of aleurioconidia is

Figure 12. A-F, I. _Malbranchea chrysosporoidea_ (A-D-UAMH 1032; E,F,I-1031). G,H,J-M. _Malbranchea circinata_ (G-H,J,M-1890; K,L-3589). Figs. 12A-12D. Colonial morphology after 21 days. A and C on PYE, B and D on cereal. C and D at 37 C, others at 25 C. Figs. 12E-12F. Aleurioconidia and intercalary arthroconidia. Figs. 12G-12H. Colonial morphology after 21 days at 25 C. G on PYE, H on cereal. Fig. 12I. Lateral or terminal aleurioconidia and intercalary arthroconidia. Figs. 12J-12M. Coiled sporogenous hyphae. Colonies x 0.9, others x 830.

[illegible] E,F, I. [illegible] (UAMH [illegible]). G,H,J-M. Malbranchea circinata [illegible] [illegible] days. A and C on PYE, B and D on cereal. C and [illegible] C, others at [illegible] C. Figs. 12E-12F. Aleurio[illegible] [illegible] intercalary arthroconidia. Figs. 12G-12H. [illegible] [illegible] after 21 days at 25 C. [illegible] [illegible] terminal a[illegible] [illegible]conidia. Figs. 12J-12M. [illegible] [illegible] colonies x 0.9, others x [illegible]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characteristic of Chrysosporium, arthroconidia may predominate in degenerate strains. The aleurioconidia of Chrysosporium are mostly subglobose, pyriform or clavate, and wider in diameter than the supporting hypha. Though this distinction appears somewhat arbitrary, it is difficult to assign this species with assurance to either Malbranchea or Chrysosporium. However, for the moment, several characters favor its placement in Malbranchea: the diameter of the aleurioconidia and intercalary arthroconidia does not exceed the diameter of the supporting hypha; intercalary arthroconidia predominate in the primary hyphae, consistent with other species of Malbranchea; the diameter of the fertile hyphae fits well within the rather narrow range for Malbranchea. Interestingly, too, vivid orange colored colonies occur in at least three other species of Malbranchea, whereas most Chrysosporium colonies are white or light colored, green, brown, grey, buff, occasionally pale orange or orange-buff.

## Habitat and Activities

Recorded from soil, dung or air samples, Maryland, Utah, Hawaii, Arizona, South Carolina, California (Orr, 1970, 1972), Japan and India. One strain was recovered from omental abscesses and the spleen after experimental intraperitoneal inoculation of soil into mice.

Keratin is digested slightly or not at all. Cellophane

is not attacked.

Material Examined

From soil: UAMH 1031, Arizona, by Emmons (E5002), from Orr as 0-1524 (same strain as UAMH 2786?); UAMH 2786, omental abscesses in mice inoculated with soil, Bethesda, Maryland, by Emmons (E5002), 1958, from Kwon-Chung, National Institute of Health, Bethesda; UAMH 1032, TYPE, Arizona by Emmons (E5003), from Orr as 0-1525; UAMH 2288, bean garden, Shokuku, Japan, by G.F. Orr; UAMH 2740, India, from H.C. Gugnani, National Institute of Communicable Disease, New Delhi, as S536; UAMH 3570, S. Carolina, from Orr as 0-3517; UAMH 3876, Oahu, Hawaii by Varsavsky (9) from Orr as 0-3295; Soil isolates from Orr: 0-3224, Maui, Hawaii, by Varsavsky (7); 0-3377, California?, by Varsavsky (20); 0-3381, Kauai, Hawaii, by Varsavsky (18); 0-3398, Oahu, Hawaii, by Varsavsky (21); 0-3399, Maui, Hawaii, by Varsavsky (25); From other sources: UAMH 1060, coyote dung, Kern Co., California, by G.F. Orr as 0-837 (NRRL A-10663); UAMH 1241, plate contaminant, Edmonton, 1962; UAMH 2051, Hawaiian Islands, soil?, from Varsavsky, CDC, as KAVAI-18; UAMH 2850, wind tunnel contaminant, Utah, by G.F. Orr 1967, as 9BR5; UAMH 3856, spore mass, Dugway, Utah, from Orr as 0-3077; NRRL 6087, from J.J. Ellis

*Malbranchea circinata* Sigler et Carmichael sp. nov.

Perfect State: Myxotrichum Kunze

Description

Colonies on PYE (Fig. 12G) are slow growing (29 mm in 21 days), pale creamy yellow, with floccose tufts of hyphae at the center surrounded by a wide (4-8 mm) band of slimy tufted surface growth. The reverse is yellow.

On cereal, colonies (Fig. 12H) attain the same diameter, but growth is more luxuriant, dense, velvety, smooth with a narrow slimy margin. The color is dark gold, reverse yellow. Droplets of brown exudate appear near the center and extend to the periphery, gradually drying and leaving the surface pitted. A reddish brown pigment diffuses into the medium extending well beyond the margin of the colony by 21 days.

There is no growth at 37 C and growth at 18 C and 30 C is slower than at 25 C.

The sporogenous hyphae (Figs. 12J-12L), arising as lateral branches somewhat broader in diameter than the narrow vegetative hypha which bears them, are tightly coiled or curved, closely spaced, eventually forming a dense cluster (Fig. 12M). Arthroconidia are smooth, cylindrical, curved, kidney shaped or ovate, almost discoid in end view, pale yellow, or yellowish brown, in age purple or dark yellow-green, 2-3um x 3-5um. No arthroconidia develop on

the primary hyphae.

Cleistothecia (Fig. 13A) are dark brown, almost black, discrete, 350-450um in diameter, excluding appendages, spherical but tending to coalesce, not easily dislodged from the conidiogenous hyphae. Peridial hyphae are dark brown, thick walled, smooth, continuously branched, terminating in 'antler-horn' appendages (Figs. 13A,13C-13E) with narrow delicate branchlets (Figs. 13C,13D), pointed toward the perimeter. Appendages of a second type are uncinate (Figs. 13A,13C), sparingly septate, thick, smooth walled, 100-200um long, 5-7um wide at the tip and the same diameter or slightly narrower at the base. The uncinate appendages arise at the center or near the periphery from a bifurcate base with the other branch terminating in the 'antler-horn' appendage (Fig. 13C).

No ascospores were found. When the cleistothecium is crushed, yellow-green or purple coiled conidiogenous hyphae in the centrum give the impression of asci, and the mature kidney shaped or ovate conidia can be mistaken for ascospores. However, the size should distinguish the arthroconidia.

Holotype: soil, Dugway, Utah, UAMH 1890 (0-1103, DPG 111)

## Discussion

The formation of cleistothecia corresponded to sectoring of the colonies on oatmeal agar at 25 C

Figure 13. A-E. _Malbranchea circinata_ (A-UAMH 3589; B-E-1890). F-J. _Malbranchea flavoroseus_ (F-H-1051; I,J-1065). Fig. 13A. Cleistothecium bearing uncinate appendages, x 210 BF. Fig. 13B. Sectoring on oatmeal agar after 35 days at 30 C, x 1.0. Fig. 13 C. 'Antler-horn' and uncinate appendages, x 660. Fig. 13D. 'Antler-horn' appendages, x 1320 BF. Fig. 13E. Coalescing peridial hyphae appendages of both types, x 210 BF. Figs. 13F-13G. Colonial morphology after 21 days at 25 C, x 0.9. F on PYE, G on cereal. Figs. 13H-13I. Curved fertile hyphae bearing alternate arthroconidia, x 830. Fig. 13J. Cleistothecia (minute black specks) formed at the center and near the periphery after 42 days at 25 C on oatmeal agar, x 0.9.

Figure [illegible]. A-[illegible]. *[illegible]ranchea* *circinata* (A-UAMH [illegible] [illegible]). F-J. *[illegible]anchea* *flavoroseus* (F-H-1051; I, [illegible]). Fig. [illegible]. [illegible]stothecium bearing uncinate [illegible] 2[illegible]. Fig. 13B. Sectoring on oatmeal agar after [illegible] at [illegible] C, x 1.0. Fig. 13 C. 'Antler-horn' and u[illegible] [illegible]ages, x 660. Fig. 13D. 'Antler-horn' app[illegible] [illegible]. Fig. 13E. Coalescing peridial hyphae appendag[illegible] [illegible] [illegible] after 21 days at 25 C, x 0.9. F on PYE, G on [illegible]. [illegible] 13H-13I. Curved fertile hyphae bearing alternate [illegible]dia, x 830. Fig. 13J. C[illegible] ([illegible] black [illegible]) formed at the center and near the periphery after [illegible] days at 25 C on oatmeal agar, x 0.9.


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(Fig. 13B). Indeed, cleistothecia consistently formed along the interface of the sector, which was white with sparse aerial growth and glabrous margin, compared to the remainder of the colony which was yellowish brown, powdery and dense with abundant conidial formation. Transfers from the sector to fresh oatmeal agar produced a colony degenerate in appearance with scant aerial growth and pale color. Coiled conidiogenous hyphae were less abundant. Transfers from the powdery yellow brown portion yielded colonies similar in appearance in which sectors eventually appeared. Asexual sporulation was profuse in the powdery areas. A transfer of a single cleistothecium yielded again a sectoring colony developing cleistothecia along the interface. Formation of cleistothecia in sectors has also been recorded for atypical colonies of _M. stipitatum_ (Orr _et al_., 1963b).

Attempts to stimulate ascospore production by mating were unsuccessful, although only two isolates were available. Both isolates required the presence of the cellophane membrane for cleistothecia to occur. None were found on the agar alone.

It is difficult to relate this species to any _Myxotrichum_ species already described, especially in the absence of ascospores. Our cultures of _M. carminoparum_ Robak (Orr _et al_., 1963b) (UAMH 1597 and 1906) also have failed to produce ascospores, but this species differs in its conidial state, lacking the coiled conidiogenous hyphae

of *M. circinata*, and in its colonial morphology. At 25 C, the colony of the type strain (UAMH 1597, CBS 224.31) is sparse and restricted and the observation of Orr *et al.* (1963b) of growth at 7 C ± 2 C suggests a lower optimum temperature for growth.

The peridial hyphae of *M. chartarum*, 'witches-broom-like' in appearance (Orr *et al.*, 1963b) is somewhat similar but the terminal branchlets are more spiny and less delicate in appearance. Here again, the coiled conidiogenous hyphae are absent, though *M. chartarum* has an arthroconidial imperfect state.

## Habitat and Activities

Recorded only from soil, Utah (Orr and Kuehn, 1972).

*M. circinata* digests cellophane but fails to attack keratin. Hairs sprinkled on DSA become purple in color after three weeks from the diffused pigment.

## Material Examined

UAMH 1890, TYPE, soil, Dugway, Utah, from Orr as O-1103 (DPG 111); UAMH 3589, soil, Terra, Utah, from Orr as O-3225 (DPG 118)

## *Malbranchea flavoroseus* Sigler et Carmichael sp. nov.

Perfect state: *Myxotrichum* Kunze

Description

Colonies on PYE grow moderately slowly (29-32 mm in 21 days) adhering poorly to the cellophane so that the margin lifts up and curls toward the center to form a distinct petaloid pattern (Fig. 13F). The margin is lobate with new growth extending unevenly beneath the raised perimeter. Colonies are creamy white, buff in the center, reverse dark tan in the center, yellow at the periphery, velvety.

On cereal, colonies (Fig. 13G) are the same diameter, flatter, pinkish buff in color and floccose. A dark brown pigment diffuses into the agar below the colony.

On oatmeal agar, colonies (Fig. 13J) become deep rose pink in the center with paler pink margin, floccose, crateriform. Growth is optimum at 25 C with slightly slower growth at 18 C and 30 C. Cleistothecia form most abundantly at 18 C, developing at the center and close to the periphery (Fig. 13J).

There is no growth at 37 C; the optimum range is 25-30 C.

Arthroconidia developing on short, loosely curved or arcuate lateral branches (Figs. 13H,13I) are cylindrical, often curved, hyaline, smooth, 1.5-2um x 3-5um. In age, arthroconidia become swollen, rounded or subglobose.

Cleistothecia are spherical, approximately 400-600um, discrete, black (Fig. 14A), composed of a network of smooth, thick walled, septate dark brown peridial hyphae, 5-6um in diameter, having blunt tips and lateral bluntly pointed spines along the length deflexed at an acute angle toward the center (Fig. 14B). The blunt tipped ends of the peridial appendages extend 120-200um beyond the densely branched center (Fig. 14A). No other appendages are formed. Asci and ascospores were not observed.

Holotype: soil, Riverside County, California, by G.F. Orr, UAMH 1051 (O-643)

Discussion

Neither of the two strains studied developed ascospores even after prolonged growth and multiple transfers. Attempts to cross the two isolates were unsuccessful. A third strain (UAMH 3909, NRRL 6088) received late in the study also failed to develop ascospores. No isolation data was provided with this strain, but it was deposited by G.F. Orr in NRRL. It could be the same strain as either UAMH 1051 or 1065.

The perfect state of this species is assigned to *Myxotrichum* primarily on the basis of its dark brown peridial hyphae. In the absence of ascospores, a specific epithet has not been chosen, nor can the isolates be assigned with certainty to any known species. Although

superficially resembling *Myxotrichum deflexum* Berkeley (Ann. Nat. Hist. 1:260, 1838), this species differs in several respects. First, the cleistothecia are much larger, 400-600um, compared to 100-400um for *M. deflexum* (Orr *et al*, 1963b; Apinis, 1964); the appendages of M. *deflexum are* narrow 1.4-1.8um (Orr *et al*., 1963b) or up to 3um (Apinis, 1964) in diameter with lateral branches, deflexed either upward or downward, having hyaline blunt apices, whereas those of this species are wider, 5-6um in diameter, with lateral bluntly pointed spines, brown in color, branched consistently downwards (Fig. 14B); finally, a conidial state has not been observed for *M. deflexum* (Orr *et al*., 1963b; Apinis, 1964).

Other characters of this species are also common to *Myxotrichum*. Orr *et al*. (1963b) reported cessation of growth at 37 C for all *Myxotrichum* species, characteristic reddish brown diffusing pigments, and pink or reddish colored colonies for some species. Pink or reddish colored colonies are notable in *M. aeruginosum*, *M. deflexum* and *M. stipitatum*. In addition, this species is cellulolytic, as are other species of *Myxotrichum*.

## Habitat and Activities

The two isolates were recovered in California, one from soil, the other from lung of kangaroo rat.

Both strains are cellulolytic after several weeks.

Material Examined

UAMH 1051, TYPE, soil, Riverside Co., California, by G.F. Orr as 0-643; UAMH 1065, lung, kangaroo rat, Palo Verde, California, from Orr as 0-647; UAMH 3909 (NRRL 6088)

*Malbranchea pulchella* Saccardo et Penzig 1882

Michelia 2:638

Synonyms

= *Malbranchea bolognesii-chiurcoi* Vuillemin, Pollacci et Nannizzi 1925, in Bolognesi et Chiurco, Archivi di Biologia 1:255-276

= *Actinomyces bolognesii-chiurcoi* (Vuill. *et al*.) Dodge 1935, Medical Mycology p. 766

= *Malbranchea kambayashii* Kambayashi 1934, Archiv fur Dermatologie u. Syphilis 170:97-106

History

Saccardo (1882) described *Malbranchea* with a single species *M. pulchella* Saccardo et Penzig, reiterating his description in 1886. Saccardo (1908) and Saccardo and Trotter (1913) reduced Miehe's (1907) thermophilic *Thermoideum sulfureum* to synonymy with *M. pulchella*.

Two other species were described, *M. bolognesii-chiurcoi* Vuillemin, Pollacci and Nannizzi in Bolognesi and

Chiurco (1925) and _M. kambayashii_ Kambayashi (1934). Though we have not seen the original descriptions, Baldacci, Ciferri and Vaccari (1939) reviewed both and reduced them to synonymy with _M. pulchella_ based on a comparison of cultural and microscopic characteristics of four isolates: _M. pulchella_ of Buisman, _M. bolognesii-chiurcoi_ of Bolognesi and Chiurco (1925) and Rivelloni (1938), and _M. kambayashii_ of Kambayashi (1934). With the exception of the Rivelloni strain which showed scant growth, all failed to grow at 37 C. Furthermore, the authors could find no reason to justify Dodge's (1935) transfer of _M. bolognesii-chiurcoi_ to _Actinomyces_.

Elisei (1940a) in a similar comprehensive examination of _Malbranchea_, reviewed the literature and also studied the same four isolates. Elisei, revising the genus, concurred with Saccardo (1908) in placing _T. sulfureum_ in synonymy with _M. pulchella_. Elisei also observed a number of microscopic features not previously recorded including nodular organs, spiral hyphae, and pectinate bodies. The latter were reviewed again by Elisei (1940b) in comparison to some dermatophytes.

Few reports of other _Malbranchea_ species are cited in the literature. In 1913, Sumstine transferred _Rhinotrichum pulveraceum_ Ellis to _Malbranchea_ as _M. pulveracea_. Linder (1942) disagreed and proposed the combination _Oidium pulveraceum_ because the conidia were borne in chains on

short denticles. Clamp connections on the hyphae indicate that this species is a conidial state of the genus *Botryobasidium* of the Basidiomycotina or a related genus.

Cooney and Emerson (1964) studying a thermophilic isolate of *Malbranchea* proposed a new variety *M. pulchella* var *sulfurea*. However a number of characters differentiate the mesophilic *M. pulchella* from the thermophilic isolates suggesting that the latter should be included in a separate species.

More recently, Wang (1965) again described and illustrated a mesophilc *Malbranchea pulchella* isolate from a pulp sample.

Von Arx (1970) stated that *Malbranchea* included 3 or 4 species, but named only *M. pulchella*.

## Description

Colonies on PYE (Fig. 14C) growing moderately slowly (26-33 mm in 21 days) are flat, or adhere poorly to the cellophane membrane, so that the center or the margin detaches and lifts up with new growth extending below. The margin is entire, or lobate and undulate. Colonies are white or mostly tan or pale brown, reverse tan or yellow, downy or velvety in texture. Creamy white exudate droplets occasionally form on the surface.

On cereal, colonies (Fig. 14D) attain a similar

diameter (20-30 mm in 21 days) but remain more firmly attached to the cellophane. In appearance, colonies are tan, white or buff, reverse pale or dark tan or yellow, flat with a central umbo and few outward radiating folds, or dome shaped, downy or velvety. Droplets of white or brown exudate sometimes form on the surface, and a brown pigment diffuses into the agar below.

Good growth occurs at 30 C but there is no growth at 37 C.

Fertile hyphae, arising as short lateral branches from the narrow (1-2um wide) vegetative hyphae form multiple branches which are tightly coiled, arched, or curved (Figs. 14E-14H). Arthroconidia are cylindrical, curved, hyaline, in mass tan, smooth 1.5-3um x 2-6.5um, mostly 1.5-2.5um x 2.5-5um. Accessory arthroconidia are rarely formed by segmentation of the primary hyphae.

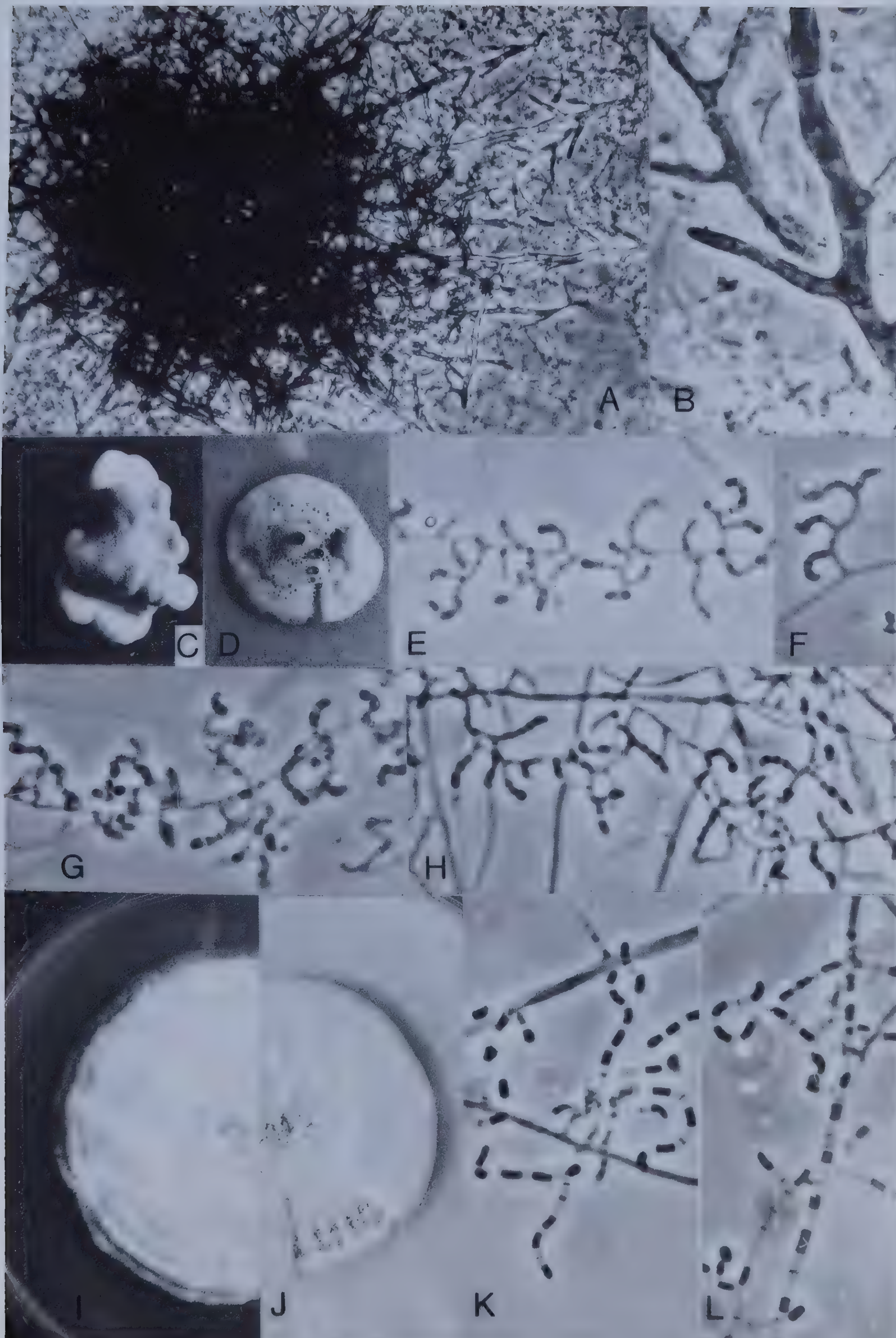
Typical strain: from pulp sample at paper towel mill, N. Y. State, 1959, by C.J.K. Wang, UAMH 1560

## Discussion

Considerable variation was initially evident among isolates received as _M_. _pulchella_. Based on Saccardo's measurement of the conidia as 3 x 2.5um as well as examination of photomicrographs from a slide (DAOM 41374) of the type, some of these isolates have been excluded (see _Malbranchea_ _flocciformis_ and _M_. _flavoroseus_). Arthroconidia

Figure 14. A-B. _Malbranchea flavoroseus_ (UAMH 1051). C-H. _Malbranchea pulchella_ (C,D,F,G,-1560; E,H-3189). I-L. _Malbranchea_ state of _Auxarthron conjugatum_ (I,J-3817; K,L-3156). Fig. 14A. Cleistothecium composed of dark brown peridial hyphae. Fig. 14B. Peridial hypha with deflexed lateral spines. Fig. 14C-14D. Colonial morphology after 21 days at 25 C. C on PYE, D on cereal. Fig. 14E-14H. Coiled or curved sporogenous hyphae. Fig. 14I-14J. Colonial morphology after 21 days at 25 C. I on PYE, J on cereal. Fig. 14K-14L. Alternate arthroconidia of curved branched and straight primary hyphae. Colonies x 1.0, others x 830, except A, x 210.

Figure 14. A-B. *Malbranchea flavoroseus* (UAMH [illegible]). [illegible] *Malbranchea* [illegible] (C,D,F,G,-1560; E,H-3189). [illegible] [illegible] 3156). Fig. 14A. Cleistothecium composed of [illegible] peridial hyphae. Fig. 14B. Peridial hypha with [illegible] lateral spines. Fig. 14C-14D. Colonial morpholo[illegible] 21 days at 25 C. C on PYE, D on cereal. Fig. 14E-14[illegible] [illegible] hyphae. Fig. [illegible] Colonial morphology after 21 days at 25 C. I on PYE, J [illegible] cereal. Fig. 14K-14L. Alternate arthroconidia of c[illegible] [illegible] and straight primary hyphae. Colonies x [illegible] [illegible] x 830, except A, x 210.


A
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of DAOM 41374 measure 1.5um x 2-4um. Of those remaining, UAMH 3794 (strain Kambayashi) differs from the others in forming slightly larger arthroconidia (2.5-3um x 4.5-6um) and wider primary hyphae (3um wide) which fragment to form accessory arthroconidia. However, this strain resembles the others in colonial morphology and failure to grow at 37 C.

UAMH 3783 (strain Rivelloni) failed to grow at 37 C although Baldacci *et al*. (1939) reported slight growth. Also the size of the arthroconidia, 2.5-3um x 3.5-6.5um differs slightly from the measurements given by them (3-4um x 6.5-7.5um), but variations could be expected in view of the prolonged maintenance of this isolate. Indeed, Cooney and Emerson (1964) reported no sporulation at all. In UAMH 3907 (strain *M. bolognesii-chiurcoi*), production of arcuate fertile hyphae was weak. However, this strain was initially contaminated with bacteria and at first, failed to sporulate at all. When freed of contamination, sporulation occurred after prolonged growth on cereal agar. However, this strain appears to be somewhat degenerate. Baldacci *et al*. (1939) reported arcuate hyphae in the type strain of *M. bolognesii-chiurcoi*.

None of these three strains corresponds exactly with the typical strain. However, all three more closely resemble *M. pulchella* than any other species described herein.

In general, the following characters distinguish *M*.

*pulchella* from other species: buff or tan slow growing colonies, no growth at 37 C, lack of accessory arthroconidia and presence of cellulolytic activity.

## Habitat and Activities

Occurrence: Reported from cardboard (Saccardo, 1882) and pulp (Wang, 1965), USA, wallpaper, and human sources in Italy, China, Sardinia (Baldacci *et al.*, 1939; Cooney and Emerson, 1964). Hubalek, Balat, Touskova and Vik (1973) and Hubalek (1974a,b) isolated *M. pulchella* from nests and feathers of birds in Czechoslovakia and Yugoslavia.

Pathogenicity: Cooney and Emerson (1964) in discussing the pathogenicity of the human isolates noted that experimental inoculation by Vuillemin, Pollacci and Nannizzi into white rats, and by Kambayashi into guinea pigs reaffirmed the pathogenicity of their isolates. However, similar experiments by Rivelloni were unsuccessful in demonstrating pathogenicity.

Activity: *M. pulchella* digests cellophane after several weeks. A single strain (UAMH 3189) also digested keratin slightly with single hyphae penetrating the hair.

## Material Examined

UAMH 643 and 1191, photomicrographs of slide (DAOM 41374) of TYPE, wet cardboard; UAMH 1560, from pulp sample at a paper

towel mill, N.Y. State, 1959, by C.J.K. Wang, State University of N.Y., Syracuse; UAMH 3189, card, Gravesend, England, by P. Russell (BS421), 1954, from CMI as 57206; UAMH 3783, CBS 204.38, from Ciferri as 'ceppo Rivelloni'; UAMH 3794, from CBS 203.38, from Ciferri as _M. kambayashii_, 1938; UAMH 3909, from CBS 202.38, from Ciferri as _M. bolognesii-chiurcoi_, 1938

_Malbranchea_ state of _Auxarthron conjugatum_ (Kuehn) Orr et Kuehn in Orr _et al_. 1963a, Can. J. Bot. 41:1452

## History

Orr _et al_. (1963a) transferred _Myxotrichum conjugatum_ Kuehn (1955b) to _Auxarthron_. De Vroey (1970) referred to a new species of _Auxarthron_ isolated from soil in Burundi and his fig. 39 indicates a resemblance to _A. conjugatum_.

## Description

Colonies on PYE (Fig. 14I) grow moderately rapidly (46-59 mm in 21 days) and are pale yellow orange or orange-buff, reverse dark orange, dry, powdery, cracked or powdery near the center with downy, creamy white overgrowth and periphery. If mostly powdery, the colony is flat with central downy umbo, otherwise zonate with 7-8 outer zones including the margin.

On cereal, colonies (Fig. 14J) are orange-buff in

color, flat, powdery or with downy creamy white to pale orange pleomorphic overgowth, reverse light to dark orange. Cleistothecia form near the center of the colony.

Growth at 37 C is slow (7-9 mm at 14 days); the colonies are pale orange, heaped up, downy, or velvety, reverse tan or orange.

Arthroconidia borne on narrow lateral arcuate branches (Figs. 14K,14L) are cylindrical, curved or straight, smooth, hyaline, in age pale orange, 1.5-2um x 2.5-5um. Arthroconidia formed by segmentation of the broader, straight hyphae (Fig. 14L) are 3 x 3-5um in diameter, often becoming swollen, rounded or subglobose in age (Fig. 15A).

This species is homothallic, and the perfect state has been described in detail by Orr and Kuehn (in Orr _et al_., 1963a).

## _Discussion_

In early stages of growth, the _Malbranchea_ state of _Auxarthron conjugatum_ closely resembles _M_. _aurantiaca_. The _Malbranchea_ states are difficult to differentiate, both forming orange colored colonies, and having arcuate fertile hyphae and arthroconidia of a similar size range. Indeed, in order to determine the relationship between the two species, strains of _M_. _aurantiaca_ were crossed, and single ascospore isolations of several _A_. _conjugatum_ strains were

attempted. However, single ascospore isolates from five strains (UAMH 3130, 3156, 3516, 3517, and 3519) of _A_. _conjugatum_ were consistently self-fertile. The sexual crosses of _M_. _aurantiaca_ were negative with the exception of one cross, UAMH 1707 x 3705 (Table III) which formed a single cleistothecium having elongate appendages and containing few spiny, oblate ascospores (see _Malbranchea aurantiaca_).

In their original description of _Auxarthron conjugatum_ Orr and Kuehn (1963a) noted that elongate appendages were straight, hooked or coiled, rarely branched. However, apical branching of elongate appendages (Fig. 15B) occurred frequently in all strains of _A_. _conjugatum_ when cultured on a variety of media, including cereal, oatmeal and DSA agar. A single cleistothecium, in addition to branched elongate appendages, also bore some which were unbranched, straight or hooked at the apex.

Orr and Kuehn (1963a) reported that ascospores of _A_. _conjugatum_ were spherical or ovoid, asperulate, smooth when young but becoming asperulate in age. Our examination indicates that the ascospores are oblate (Figs. 15C,15D), 1.5-2.2um x 2.5-3.2um and very finely roughened. In all strains examined, the asperulate nature of the ascospores is almost beyond the resolution of the phase contrast light microscope. Even at high magnification (x2700), the finely roughened edge of the ascospore is barely discernible

(Figs. 15C,15D). Scanning electron microscopy would be required to resolve the exact nature of the ascospore surface.

Habitat and Activities

Recovered from soil, dung, feathers and lungs of rodents, in the USA, from Arizona, California, Kansas, and from Australia, Mexico, India and the Sudan (Orr et al., 1963a).

A. conjugatum is keratinolytic, producing slight to moderate digestion of hairs without the aid of penetrating bodies. Cellophane is not attacked.

Material Examined

From soil: UAMH 3156, TYPE, Arizona, by C.W. Emmons (47), from R.K. Benjamin, RSABG as 1530 (NRRL 1244, 0-520); UAMH 3516, Sudan, received from L. Ajello, CDC as 32 b; UAMH 3517, Sudan, from L. Ajello, CDC as 32 a; UAMH 3519, Sudan, from L. Ajello, CDC, as 28 a; UAMH 3817, India, by Ghosh, from Orr as 0-1236; UAMH 3874, Kansas, from Orr as 0-3750; From other substrates: UAMH 3130, lizard dung, Chihuahua, Mexico, by R.K. Benjamin, 1964, RSABG as 1474; UAMH 3841, feathers (domestic fowl), Australia, by Rees, from Orr as 0-3153 (F91)

Malbranchea sulfurea (Miehe) Sigler et Carmichael

Figure 15. A-D. <u>Auxarthron conjugatum</u> (A,B,D-UAMH 3156; C-3817). E-J. <u>Malbranchea sulfurea</u> (E,I,J-2485; F,G-3748; H-2486). K-M. <u>Malbranchea dendritica</u> (2731). Fig. 15A. Enlarged arthroconidia and curved sporogenous hyphae. Fig. 15B. Apical branching of elongate peridial appendage. Figs. 15C-15D. Oblate, finely asperulate ascospores. Fig. 15E. Alternate arthroconidia borne on curved or coiled branches. Figs. 15F-15G. Colonial morphology after 7 days at 45C. F on PYE, G on cereal. Fig. 15H. Curved sporogenous hyphae. Fig. 15I. Thick walled chlamydospore. Fig. 15J. Thick walled mature arthroconidia. Figs. 15K-15L. Colonial morphology after 21 days at 25 C. K on PYE, L on cereal. Fig. 15M. Branching at an acute angle of fertile hyphae. Colonies x 1.0, others x 830, except C and D, x 2300.

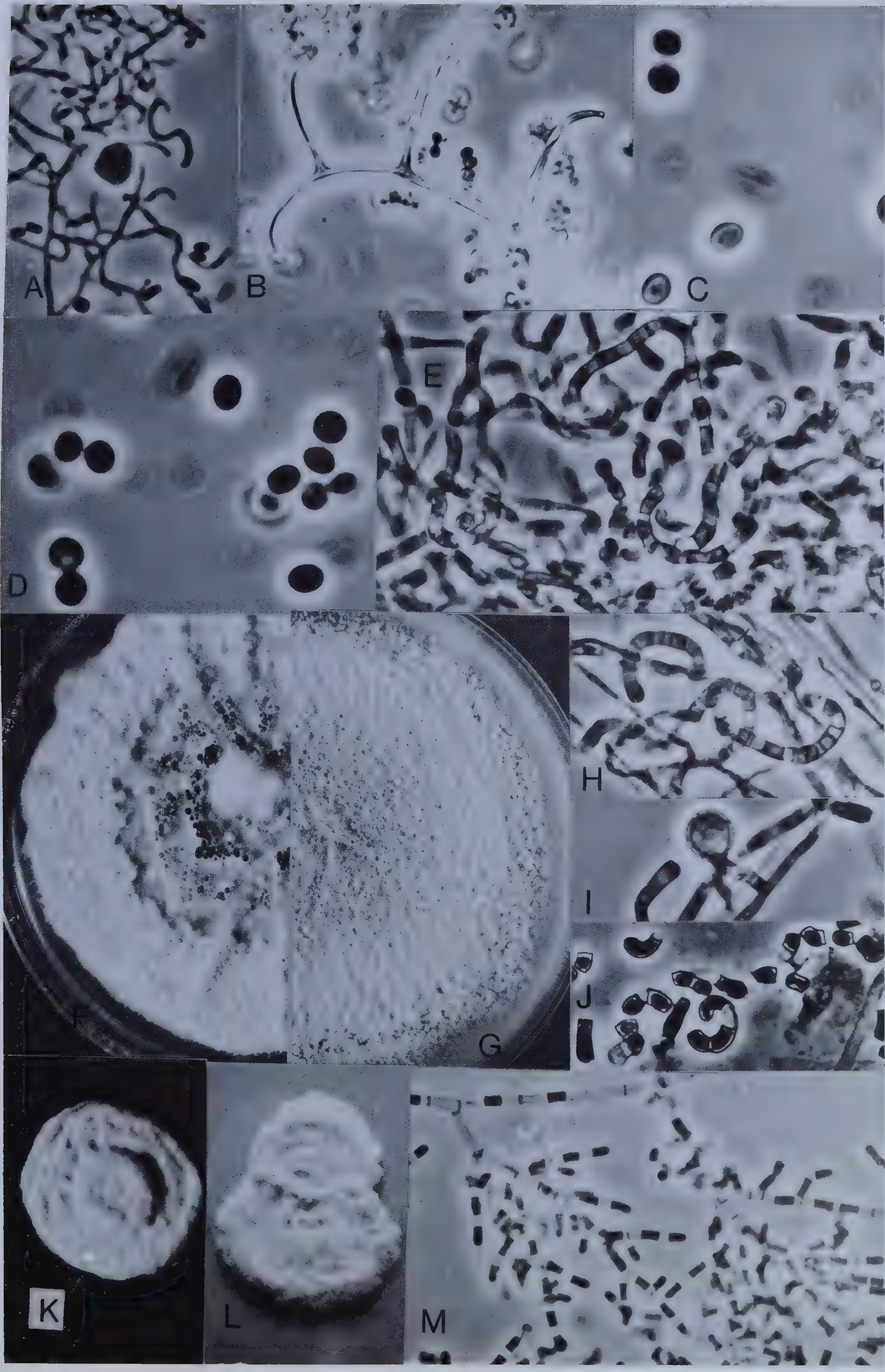
[illegible]

[illegible] 7). E-J. Malbranchea sulfurea (E,I,J-2485; F,G-3[illegible] 2486). K-M. Malbranchea dendritica (2731). Fig. [illegible] enlarged arthroconidia and curved sporogenous hyphae. Fig. [illegible]

Figs. 15C-15D. Oblate, finely asperulate ascosp[illegible] Fig. [illegible] alternate arthroconidia borne on curved or co[illegible] [illegible]

[illegible] [illegible]nous hyphae. Fig. 15I. Thick walled chlamydospo[illegible] [illegible]

[illegible] [illegible]al. Fig. 15M. Branching at an acute angle [illegible] hyphae. Colonies x 1.0, others x 830, except C and D, x [illegible].


A
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comb. nov.

Synonyms

= *Thermoideum sulfureum* Miehe 1907, Deutsche Botanische Gesellschaft 25:515 (Basionym)

= *Malbranchea pulchella* var *sulfurea* (Miehe) Cooney et Emerson 1964, Thermophilic Fungi p. 102

History

In 1907, Miehe created a new genus and species *Thermoideum sulfureum* for a fungus he found growing on spontaneously heated compost heaps. By studying the fungus in culture, Miehe not only established the thermophilic nature of the organism but also accurately illustrated and described the formation of conidia. According to Miehe, the curved fertile hypha divided itself into short segments, which later developed an internal thickened membrane to form the spore. In the process, not all the cells became spores, but only alternate ones. Saccardo (1908) and Saccardo and Trotter (1913) reduced *Thermoideum sulfureum* to synonymy with *M. pulchella* without regard for the thermophilic nature of the organism.

Elisei (1940a) examining four mesophilic strains, followed Saccardo (1908) in placing *T. sulfureum* in synonymy with *M. pulchella*.

In 1964, Cooney and Emerson reviewed the history of

*Malbranchea* and provided an excellent account of the morphology of a thermophilic strain isolated by Emerson in 1945. Their fungus corresponded in appearance to the description of the thermophilic *Thermoideum sulfureum* of Miehe (1907). A comparison with the study of Baldacci *et al*. (1939) of four mesophilic isolates indicated that these organisms differed at least in temperature relations. Cooney and Emerson examined a single mesophilic strain (strain Rivelloni) which failed to sporulate and they were unable to make a definite distinction between the thermophilic and mesophilic isolates. Therefore they proposed a new variety for the thermophilic isolates, *Malbranchea pulchella* var *sulfurea* (Miehe) until a more comprehensive comparison of the mesophilic and thermophilic strains could be made.

An examination of a number of isolates indicated that the thermophilic strains are distinct from the mesophilc *M. pulchella* and therefore a new combination is proposed here.

## Description

At 45 C, colonies (Fig. 15F) on PYE grow rapidly and robustly, almost or completely filling the petri dish by 7 days, and becoming dense and thick, smooth or with few outward radiating folds, velvety with coarse, creamy yellow tufts of hyphae over the surface. The color is sulfur yellow or yellowish tan, with yellow or pink margin, turning

by 21 days to a dark gold or buff brown or deep reddish brown color. Large droplets of dark brown exudate appear on the surface by 7 days (Fig. 15F), gradually drying and leaving the surface of the colony pitted and mealy in appearance. The medium turns dark brown or black from diffused pigment. At 37 C, growth is slightly slower (70-78 mm in 7 days) characteristically sulfur yellow in color with darker tan center, and otherwise similar in appearance to colonies at 45 C. Pigment production is slightly reduced at 37 C.

At 45 C, colonies (Fig. 15G) on cereal attain a diameter of 79-81 mm by 7 days, and are sulfur-yellow, flat, coarse, and mealy with tufts of yellow hyphae. Initial yellow exudate droplets later turn dark brown and when dried, leave the colony pitted. By 21 days, the colony turns dark tan or reddish brown, in appearance a dense, thick mat, smooth often with few irregular folds, and velvety. Diffused pigment turns the medium dark brown in color. Growth at 37 C is slightly slower, but otherwise similar. The color at 21 days is dark reddish brown. Pigment production appears slightly reduced.

Degenerate or less robust strains (UAMH 2481, 2486, and CBS 423.54) are greenish grey or drab grey in color, glabrous, hairy or leathery with scant aerial growth. The pigment exceted into the medium is much reduced.

The arthroconidia are borne on curved or loosely coiled

lateral branches (Figs. 15E,15H) arising from broader vegetative hyphae (3-6um in diameter). The vegetative hyphae are hyaline, later yellowish brown with prominent racket hyphae, the enlargement at the septum reaching a diameter of 9um or more. In some strains, thick walled intercalary or terminal chlamydospores (Fig. 15I) formed, increasing in abundance at 45 C. Arthroconidia (Fig. 15J) are cylindrical, often curved, thick walled, often with attached hyaline frill from the outer hyphal wall of the separating empty cell, hyaline at first, later yellow, tan or yellowish green (2)2.5-4(4.5)um x (3)3.5-7.5(8.5)um, mostly 3-4um x 4-7um.

## Discussion

Cooney and Emerson (1964) and Awao and Otsuka (1973) provide further accounts of the morphology of this species.

*M. sulfurea* differs from *M. pulchella* in its thermophilic nature, its colonial morphology, its broader vegetative hyphae and its thick-walled broader arthroconidia. In its capacity to grow at high temperature, *M. sulfurea* is distinct from all other species of *Malbranchea*. Furthermore, chlamydospores are formed regularly.

No other spore state was observed and attempts to mate the isolates by pairing them on oatmeal agar at 37 C in 3% $CO_2$ were unsuccessful.

## Habitat and Activities

Occurrence: Numerous records of *M. sulfurea* from heated substrates indicate that this species is ubiquitous and has a world wide distribution.

*M. sulfurea* has been reported from plant heaps and rotting plant material: guayale rets, California (Cooney and Emerson, 1964); composting heaps, Germany, Texas (Miehe, 1907; Rode, Foster and Schuhardt, 1947); wheat straw compost, England (Chang and Hudson, 1967) and stacked tobacco leaves, South Africa (Eicker, 1972). It has been isolated from soil in Japan (Awao and Otsuka, 1973); peanut kernals and associated soil, Texas (Taber and Pettit, 1975); coal spoil tips, England (Evans, 1971); faeces of Cape sparrow, South Africa (Eicker, 1972); dung of deer, Japan (Minoura, Yokoe, Kizima, and Nehira, 1973) and cattle, Alberta; hen-house litter, Netherlands; snuff, USA, (Tansey, 1975) and from air sample surveys, England (Hudson, 1973).

*M. sulfurea* is notably absent from self-heating wood chip piles (Shields, 1969; Tansey, 1970 (Abstract), 1971b; Smith and Ofosu-Asiedu, 1972).

Growth rates: Table IV compares maximum diameters of colonies of *M. sulfurea* tested at five temperatures. Growth occurred at 25 C, albeit slowly, and at 55 C for all isolates tested.

TABLE IV
DIAMETER (MM) OF AGAR PLATE COLONIES OF MALBRANCHEA SULFUREA AFTER GROWTH AT DIFFERENT TEMPERATURES

| Temperature (°C) | 25[1] | 30[1] | 37[2] | 45[2] | 55[2] |
|---|---|---|---|---|---|
| Time (days) | 28 | 35 | 7 | 7 | 7 |
| 2481 | + | 75 | 78 | 90 | N |
| 2485 | + | 30 | 73 | 90 | 42 |
| 2486 | + | 66 | 72 | 90 | N |
| 3747 | + | 23 | 74 | 80 | 26 |
| 3748 | + | 16 | 77 | 80 | 10 |
| 3761 | N | N | N | N | 32 |
| 3788 | N | N | N | N | 42 |
| 3789 | N | N | N | N | 25 |

Legend: + Scant growth (diameter of colony < 5 mm)
N Not tested
1 Growth tested on oatmeal agar only
2 Growth tested on PYE agar

The minimum temperature is close to 25 C, the maximum 55-57 C (Cooney and Emerson, 1964). Chapman (1974), studying the effects of temperature on growth rates of several thermophiles, concluded the optimum for M. sulfurea was 40-45 C, with no growth at 30 C or 55 C after 8 days.

Cellulolytic Activity: M. sulfurea attacks cellophane vigorously, markedly weakening the membrane by 21 days.

Chang (1967) reported growth on xylan, the major hemicellulose of straw, when provided as the sole carbon source, but no utilization of cellulose in the form of filter paper, a finding confirmed by Fergus (1969). Fergus (1969) demonstrated the use of carboxymethyl cellulose by M. sulfurea. Tansey (1971a) measured the zone of clearing of acid swollen cellulose in an agar medium. Though M. sulfurea produced a definite clearing, indicating cellulolytic activity, the zonal front was indistinct and difficult to measure. Tansey suggested that a portion of the system of enzymes required for digestion of cellulose could be lacking, thereby rendering the organism incapable of attacking insoluble cellulose.

Metabolites: Rode et al. (1947) identified an antibiotic from M. sulfurea as comparable in activity to authentic penicillin.

Ong and Gaucher (1973), examining a number of

thermophiles, characterized intracellular and extracellular proteases, one of which, the extracellular alkaline protease of _M. sulfurea_ has been defined by Voordouw, Gaucher and Roche (1974) as thermomycolase, a thermostable protease, protein in nature.

Lipids: Mumma, Fergus and Sekura (1970), studying the lipid composition of some thermophilic and mesophilic fungi, revealed a difference in the degree of saturation of fatty acids, measured by the number of double bonds per mole of fatty acid. The thermophiles, in comparison to mesophiles of the same genus, were found to be more saturated _i.e._ have fewer double bonds per mole. Crisan (1973) pointed to the higher melting point of the saturated fatty acids as a factor in the thermostability of these organisms, and discussed lipid stability as one of four hypotheses explaining thermophilism in fungi. However, Crisan considered the best explanation of thermostability resided in the basic ultrastructure of the organism, possibly in the integral genetic make-up, the cell structure or the ribosomes.

Mumma _et al._ (1970) finding a higher lipid composition for _M. sulfurea_ than some other thermophiles suggested the difference was due to extraction of the soluble pigment.

## Material Examined

UAMH 3761, NEOTYPE, from retting _Parthenium argentatum_,

Salinas, California, by Emerson (27), 1945, from CMI as 126327; From ensiling alfalfa hay forage: UAMH 2481, isolated 1953, received from Semeniuk, South Dakota State University, Brookings as 401; UAMH 2485, 1953, from Semeniuk as 422; UAMH 2486, 1953, from Semeniuk as 459; From dung: UAMH 3747, manure in cattle feedlot, Lethbridge, Alberta, by R.G. Bell, 1974, Research Station, Lethbridge as 1; UAMH 3748, manure in cattle feedlot, Lethbridge, by R.G. Bell, 1974, as 2; UAMH 3789, deer dung, Hiroshima, Japan, by K. Minoura, 1970, from Tubaki, IFO, as 9739; From other substrates: UAMH 3827, soil, hen-house litter, by A.H.M. Grimbergen, from CBS as 343.55; UAMH 3857, seed of *Oryza sativa*, China, from CBS as 115.68; UAMH 3858, from CBS as 960.72; CBS 423.54

b) *Malbranchea* species with straight fertile hyphae

*Malbranchea dendritica* Sigler et Carmichael sp. nov.

*History*

During a search for *Coccidioides immitis* in California soil, Plunkett, Walker and Huppert (1963) isolated three fungi which produced arthroconidia from acutely branched hyphae, giving a "pine-tree appearance" to the sporulation in slide culture. They inoculated three mice intraperitoneally with each strain and killed them after 10, 15 and 20 days. Small nodules were found in all mice, but

no endosporulating spherules were seen. "When planted on media, these nodules yielded colonies that were identical to the original culture. Isolates from tissue were prepared for inoculation into a second group of five mice. On the 7th day after inoculation all mice revealed endosporulating spherules at autopsy and established the identification as C. immitis. The cultures recovered from the mice were again identical to the original one." Despite the discrepancy between the results of the first and second sets of mouse inoculations, they identified their isolates as atypical Coccioides immitis.

Huppert, Sun and Bailey (1967) studied a collection of 301 strains of fungi which would grow on media containing cyclohexamide. They stated that for each strain endosporulating spherules had been demonstrated in mice inoculated intraperitoneally, and the fungus recovered in culture. Thus, they identified all their strains as C. immitis, regardless of their colonial and microscopic morphology. Details of the animal inoculations were not given. Some of their isolates (strain numbers not given) had acutely branched fertile hyphae and their Figure 6 shows the microscopic morphology which we now consider to be characteristic of M. dendritica. Their Figure 7 shows a Malbranchea similar to M. gypsea (q.v.).

Orr (1968) isolated a dendroid Malbranchea from soil. Later (Orr, 1972) he compared the pathogenicity of this

isolate (UAMH 2731, DPG-141) with several other isolates of _Malbranchea_, including strains of _M_. _gypsea_ and with _Coccidioides immitis_. Passage of each organism though mice resulted in lesions in the spleen and liver but no endosporulating spherules were observed in mice other than the ones inoculated with _C_. _immitis_. In addition, histopathological examination revealed no fungal elements, although the arthroaleuriospore formers could be recovered in culture from the tissue. _Malbranchea dendritica_ was recovered from the lungs in addition to the spleen and liver and survived four serial passages though mice. Control mice inoculated with physiological saline were negative.

It appears probable that the three isolates of Plunkett _et al_. (1963) and some of the atypical _Coccidioides immitis_ strains of Huppert _et al_. (1967) should be included in _Malbranchea dendritica_.

## Description

The colony on PYE (Fig. 15K) is moderately slow growing, reaching a diameter of 31 mm in 21 days, creamy buff, reverse buff, rising to a central plateau, velvety. On cereal (Fig. 15L), the colony reaches a diameter of 37 mm in 21 days and is white, reverse white, slightly raised with scarcely distinct zonate pattern, velvety.

Growth at 37 C is slow. On PYE, the colony is 23 mm in diameter, white, heaped up and convoluted, velvety. The

edge of the colony detaches from the cellophane membrane. On cereal, the colony is 20 mm in diameter, flat at the periphery but cerebriform in the center, waxy, pale buff in color.

The fertile hypha, branched at an acute angle from the primary hypha (Fig. 15M) is straight, bearing multiple short or long lateral branches. Branching occurs consistently at an acute angle giving the fertile hypha a tree-like appearance (Fig. 16A). Arthroconidia are first formed on the apical part of the main axis of the fertile hypha and on the lateral branches (Fig. 16B). Later, the base of the hypha and the primary hypha become regularly septate, followed by separation into arthroconidia. Arthroconidia are separated by one or more empty segments, a single segment often shorter than the length of an arthroconidium. Arthroconidia are hyaline, smooth, cylindrical 1.5-2(3) um x 3-5um.

Holotype: soil, Dugway, Utah by G.F. Orr, UAMH 2731 (DPG-141)

Discussion

The regularly acute angle of branching of the fertile hyphae distinguishes *M. dendritica* from all other species of *Malbranchea*.

Habitat and Activities

The single strain examined was recovered from soil, Utah.

*M. dendritica* is keratinolytic, producing moderate digestion of hairs without the aid of penetrating bodies.

*Malbranchea flava* Sigler et Carmichael sp. nov.

*Synonym*

?= *Malbranchea* state of *Oncocladium flavum* Wallroth 1833, Flor. Crypt. German. p. 289

*History*

In 1900, A. L. Smith, described *Gymnoascus verticillatus*, characterized by brown thick-walled verticillate peridial hyphae and globose ascospores, 2.5um in diameter, separate or in ascus-like groups. In 1963, Orr and Kuehn described *Actinodendron verticillatum* based on Smith's description and an examination of her type specimen, HERB BM, by Stockdale and Balfour-Browne. Because of its verticillate peridial hyphae, the fungus was considered distinct from *Gymnoascus* Baranetzky. Though Smith (1900, 1901) apparently did not attempt pure cultures, Orr and Kuehn (1963) reported several recent isolations of the fungus, by Szathmary in Hungary, Plunkett in California, and Stockdale in Wales. However, the verticillate hyphae observed in the original specimens could not be reproduced in culture. Also, neither asci nor ascospores were observed

in the specimens of Szathmary and Plunkett. Several fungi were isolated from Plunkett's hair bait sample (all designated OAP- 19-317), two of which had arthroaleuriospore states. Orr and Kuehn (1963) suggested that a keratin source such as hair may be required for cleistothecial development.

Apinis (1964) reviewing _Actinodendron verticillatum_, examined several specimens, including Smith's type material, Stockdale's specimen from sheep's wool (HERB IMI 100,300), an isolate of his own from a dead bird, and one from feathers placed in a damp chamber (HERB K). In the latter material, Apinis observed asci and ascospores as well as the characteristic appendages in older brown clusters formed on the feathers. Also associated was a conidial state consisting of conidia resembling the aleurioconidia of _Chrysosporium merdarium_ and the arthroconidia of _Geotrichum_ and also endogenous microconidia. Apparently, Apinis did not attempt to make pure cultures from the feathers.

Subsequently, Hughes (1968) comparing the type specimen of _Oncocladium flavum_ Wallroth, a Hyphomycete, with the type specimen, HERB BM, of _Actinodendron verticillatum_, concluded the verticillate hyphae in both were identical. In the specimen of _O. flavum_, Hughes observed neither asci nor ascospores, nor any conidia of the _Chrysosporium_ type, but reported an arthroconidial state resembling _Oidiodendron_. According to Hughes, the verticillate hyphae were

characteristic of *Oncocladium*. Since Wallroth's original description did not refer to ascospores, Hughes considered *Oncocladium flavum* to be the conidial state of *Actinodendron verticillatum*. He also suggested that *Oncocladium* could prove to be an earlier name for *Oidiodendron*.

The taxonomic position of *Actinodendron verticillatum* was complicated by Orr and Kuehn (1971) who accepted Hughes' appraisal of *O. flavum* as a Hyphomycete, and proposed rejection of *A. verticillatum* since ascospores had not been observed in any but Smith's type specimen. Apparently, they failed to examine the two specimens reported by Apinis (1964) to have ascospores. Orr and Kuehn (1971) concluded that the verticillate hyphae were structures of the Hyphomycete *Oncocladium* and that the ascospores present in Smith's type material were not part of the same fungus.

Based on these conflicting reports, it is difficult to assess the true nature of *Oncocladium*. On the one hand, there is Apinis' assertion that ascospores are present in clusters bearing verticillate hyphae, and on the other, there is Orr and Kuehn's (1971) rejection of *Actinodendron*. While both Orr and Kuehn (1963) and Szathmary (1967b) reported verticillate hyphae in keratin-baited samples, to our knowledge, neither has induced their formation in culture. However, the list of cultures of *Oncocladium flavum* examined by Orr and Kuehn (1971) includes isolates from Plunkett's hair bait sample (OAP-19-317, miscitation

OAP-19-1317) (Orr and Kuehn, 1963) as well as several arthroaleuriospore formers corresponding in appearance to isolates described as *Gymnoascus verticillatus* by Szathmary (1967a,b).

In specimens we have examined, HERB IMI 100,300 of Stockdale, and HERB IMI 100,445, a few spiny, oblate ascospores were observed in the latter. The label on the specimen indicated *Anixiopsis stercoraria* [= *Aphanoascus fulvescens*] to be present. Conidia of the *Chrysosporium* type and the appearance of the ascospores agree with *A. stercoraria*, although no cleistothecia were observed. In any case, the appearance of the ascospores did not agree with the description of Smith (1900) and Apinis (1964). No ascospores were observed in the two slides of IMI 100,300.

In both specimens, however, an arthroconidial state is present within the clusters bearing verticillate hyphae (Figs. 16C,16D). Pseudocleistothecia or peridial appendages have been observed in culture in some heterothallic Gymnoascaceae, notably *Ctenomyces serratus* (Benjamin, 1956), *Uncinocarpus reesii*, and some *Myxotrichum* and *Arthroderma* species (Varsavsky and Ajello, 1964; Padhye and Carmichael, 1973; Sekhon, Padhye and Carmichael, 1973). The peridial hyphae of the pseudocleistothecium resemble those of the fertile cleistothecium, but the structure, devoid of asci and ascospores, contains only conidia. According to Benjamin (1956), the presence of infertile cleistothecia

confused the taxonomy of *Ctenomyces serratus* Eidam for many years. Eidam described *Ctenomyces* as having two stages, a fertile cleistothecial state and a sclerotial or resting state. However, Benjamin (1956) showed that the specimen examined by Eidam contained two different fungi, the first, *Arthroderma curreyi* and the second *Ctenomyces serratus*. No ascospores were reported by Eidam for the sclerotial state but Benjamin (1956) retained the name *Ctenomyces serratus* for the fungus distinguished by the characteristic comb-like appendages.

The infrequent occurrence of ascospores in *Oncocladium* suggests that the fungus may be heterothallic requiring mating of compatible strains before fertile cleistothecia are formed. It seems probable that the verticillate hyphae arise as thick walled extensions of the vegetative hyphae, forming clusters on the natural substrate, but remaining infertile unless compatible mating types are present. In the *Malbranchea* state of *Uncinocarpus reesii* uncinate appendages, arising as extensions of the vegetative hyphae also intertwine to form a cleistothecium when compatible strains are mated.

The verticillate hypha of *Oncocladium flavum* is narrow at the base, but becomes wider at the apical or distal end, terminating in a blunt tip and occasionally extending into a hyaline septate hyphal appendage which gradually narrows to a diameter of 2-3um (Fig. 16C). The branchlets forming the

verticils are pointed at first then become blunt tipped as the apical segment detaches. In this respect, the hyphae resemble the peridial hyphae of some *Myxotrichum* species which often terminate in blunt spines, the terminal portion having broken off.

For the present, the true nature of the association of the appendages with the arthroconidial state is not definitely established. Though Hughes (1968) has suggested *Oncocladium flavum* is a Hyphomycete characterized by verticillate hyphae, it appears to us more likely that these hyphae are peridial appendages of the perfect state. If so, the name *Oncocladium* could be retained for the perfect state since the key feature of *Oncocladium*, as Hughes (1968) noted, is the verticillate hyphae. The arthroconidial state, clearly distinct from *Oidiodendron* is assigned to *Malbranchea*.

## Description

Growth on PYE (Fig. 16F) is moderately rapid with the diameter at 21 days varying from 39-61 mm. Colonies are lemon yellow with darker gold center, occasionally sulfur yellow, greenish yellow, or creamy white, powdery, rarely woolly, dense, dry, separating and lifting from the cellophane in the center and becoming undulate with folds extending to the margin. The center and outer folds develop deep cracks or splits as the colony ages. The colony

(Fig. 16E) appears zonate at first as the advancing margin extending 1-3 mm beyond the perimeter turns from white to yellow and becomes floccose.

Growth on cereal is more restricted (26-39 mm in 21 days), appearing (Fig. 16G) flat, straw yellow in color with a darker gold center, rarely white, coarse, powdery, or woolly. A wrinkled, waxy small umbo with tiny surrounding cracked furrows, develops at the center in some strains. A slight amount of yellow pigment diffuses into the agar below the colony.

30 C is close the maximum for growth, with only a single strain (UAMH 1879) showing growth after 5 weeks (10 mm). The optimum range is 18-25 C.

Arthroconidia developing on straight, branched fertile hyphae (Figs. 16H-16J), are separated from each other by one or more empty segments (Fig. 16H) which are divided by thickened septae suggesting double septae (Figs. 16H,16I). The arthroconidia are cylindrical, cuneiform if terminal, discoid in end view, hyaline, later greenish yellow, often with refractile end walls (Fig. 16K), 2-3um x 2-6um, mostly 2-3um x 2.5-5.5um. Clamydospores are rare.

No other spore state was observed.

Holotype: soil, South California, by O.A. Plunkett, 1962, UAMH 1589 (OAP 19-317)

Figure 16. A-B. Malbranchea dendritica (UAMH 2731). C-D. Oncocladium flavum (IMI 100300). F-K. Malbranchea flava (E,J,K-2864; F,G-1589; H-1956; I-1879). Figs. 16A-16B. Tree-like appearance of branched fertile hypha. Fig. 16C. Blunt tipped verticillate hypha with hyaline terminal appendage (arrows). Fig. 16D. Verticillate appendage and associated arthroconidial state. Figs. 16E-16G. Colonial morphology at 25 C. E and F on PYE, G on cereal. E at 14 days, F and G at 21 days. Figs. 16H-16J. Alternate arthroconidia of branched fertile hyphae. Fig. 16K. Arthroconidia with refractile end walls. Colonies x 1.0, others x 830.

[illegible] (UAMH [illegible]). D–E. [illegible] (IMI 100300). F–K. [illegible]

[illegible] Figs. [illegible]

[illegible] fertile hypha. [illegible]

[illegible] verticillate hypha with hyaline [illegible]

[illegible] (arrows). Fig. 16D. Verticillate [illegible] and

[illegible] arthroconidial state. Figs. [illegible] Colonial

[illegible] at 25 C. E and F on PYE, G on cereal. [illegible]

[illegible] G at 21 days. Figs. 16H–16J. Alternate

[illegible] branched fertile hyphae. [illegible]

[illegible] with refractile end walls. [illegible]

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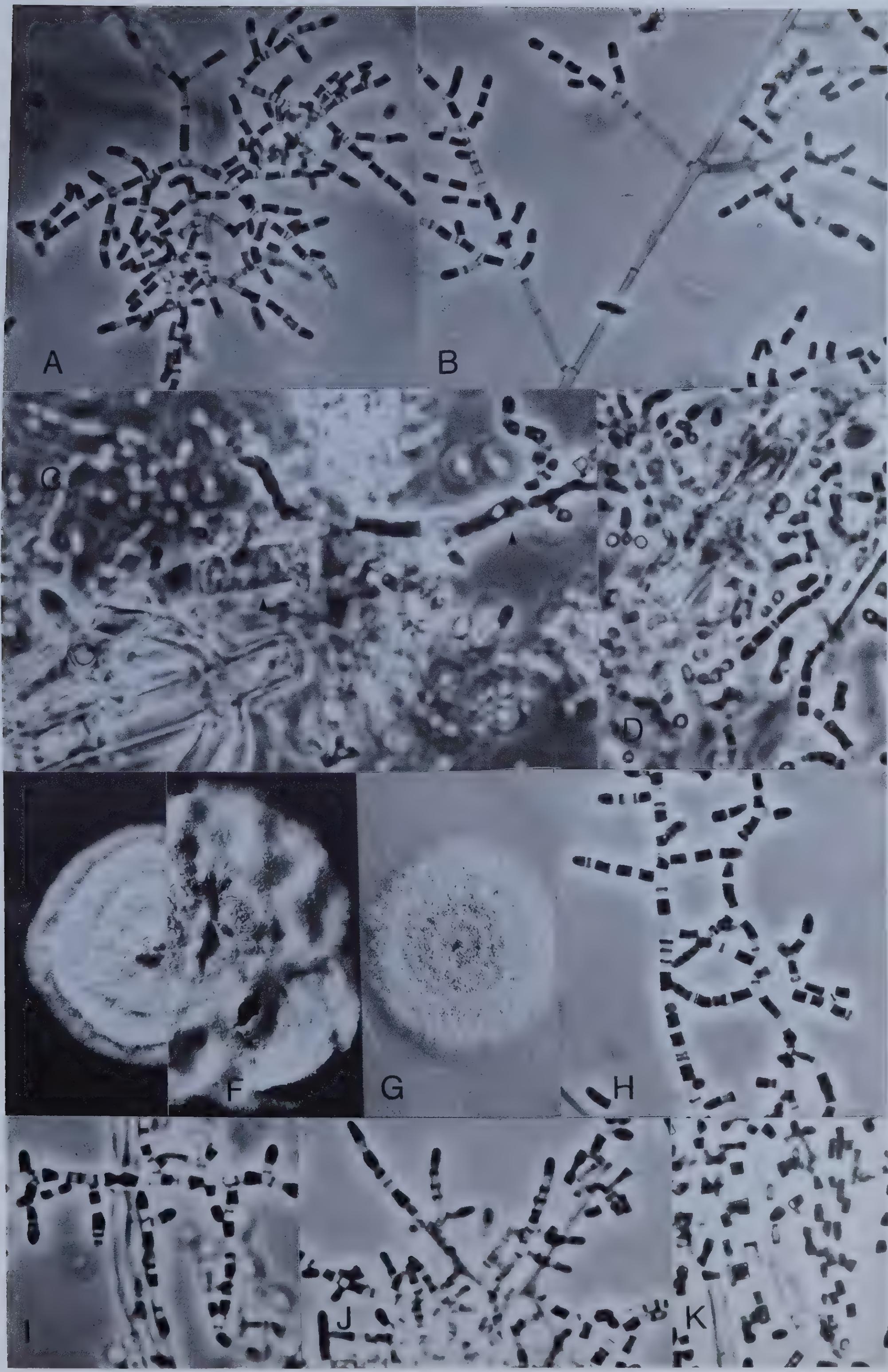

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## Habitat and Activities

Recorded from soil, California, Hungary and France (Szathmary, 1967a,b; Orr and Kuehn, 1963, 1971).

Most isolates of *M. flava* showed only slight digestion of hairs without penetration of the hair by hyphae. Two strains (UAMH 1879 and 1956) did not attack hair at all. This limited capacity to digest hair *in vitro* is interesting since most were isolated by keratin-bait techniques. In addition, the requirement for a keratin source has been suggested as a prerequisite for cleistothecial formation (Orr and Kuehn, 1963).

## Material Examined

Soil isolates received from Orr: UAMH 1589, TYPE, California, by O.A. Plunkett, 1962, OAP-19-317; UAMH 1879, California, by O.A. Plunkett, OAP-19-317(6); UAMH 1956, Argentina, Varsavsky, EV 5V (O-3596); UAMH 2859, Tunis, by De Vroey, RV 19535a (O-3496); UAMH 2860, Tunis, sector of RV 19535a, by Orr, as RV 19535b; UAMH 2864, fabric-bait, Hungary, Szathmary, 1967, O-3512; UAMH 2865, fabric-bait, Hungary, Szathmary, 1967, O-3514;
Material of *Oncocladium flavum*: HERB IMI 100,300, sheep's wool on dung, Wales, by P.M. Stockdale, 1962 (UAMH 3920); HERB IMI 100,445, wool, associated with *Anixiopsis stercoraria* (UAMH 3921)

*Malbranchea flocciformis* Sigler et Carmichael sp. nov.

## Description

Colonies on PYE (Fig. 17A) grow moderately slowly (22-42 mm in 21 days) adhering poorly to the cellophane, and lifting off the membrane at the margin or at the center with the margin depressing the medium. In appearance, colonies are domed with deep folds or convolutions, or petaloid, pale or tangerine orange, reverse tan, and downy or velvety. Exudate droplets occasionally form near the center.

On cereal agar, colonies (Fig. 17B) are somewhat flatter, (24-38 mm in 21 days) either dome shaped with the margin depressing the medium and downy, or crateriform, zonate and powdery. The color is pale or tangerine orange or pale yellow. A single strain (UAMH 675) formed yellow exudate droplets on the surface and a brown pigment below the colony.

With the exception of UAMH 3273, which showed scant growth (5 mm in 21 days on PYE) the isolates did not grow at 37 C.

Arthroconidia are borne on the primary hyphae (Fig. 17C) and on short or long lateral branches (Figs. 17C,17D) which often branch repeatedly to form a dense tuft (Fig. 17E). The fertile hyphae are characteristically straight, occasionally curved (Fig. 17F).

Arthroconidia are cylindrical, hyaline or pale yellow, orange in mass, smooth (1.5)2-2.5um x 2-5.5um.

Holotype: from briny soil, Chateau-Saline, Lorraine, France, UAMH 3273

## Discussion

Two of the strains included here were initially determined to be *M. pulchella*. Of the two, UAMH 675 resembles *M. pulchella* in its curved fertile hyphae (Fig. 17F) but differs in forming accessory arthroconidia on the primary hyphae and in having orange colored colonies. Although UAMH 675 may not be suitably placed in this species, it is retained here until further isolates are reported.

## Habitat and Activities

Recovered from briny soil, France and wood of a lemon bin, California.

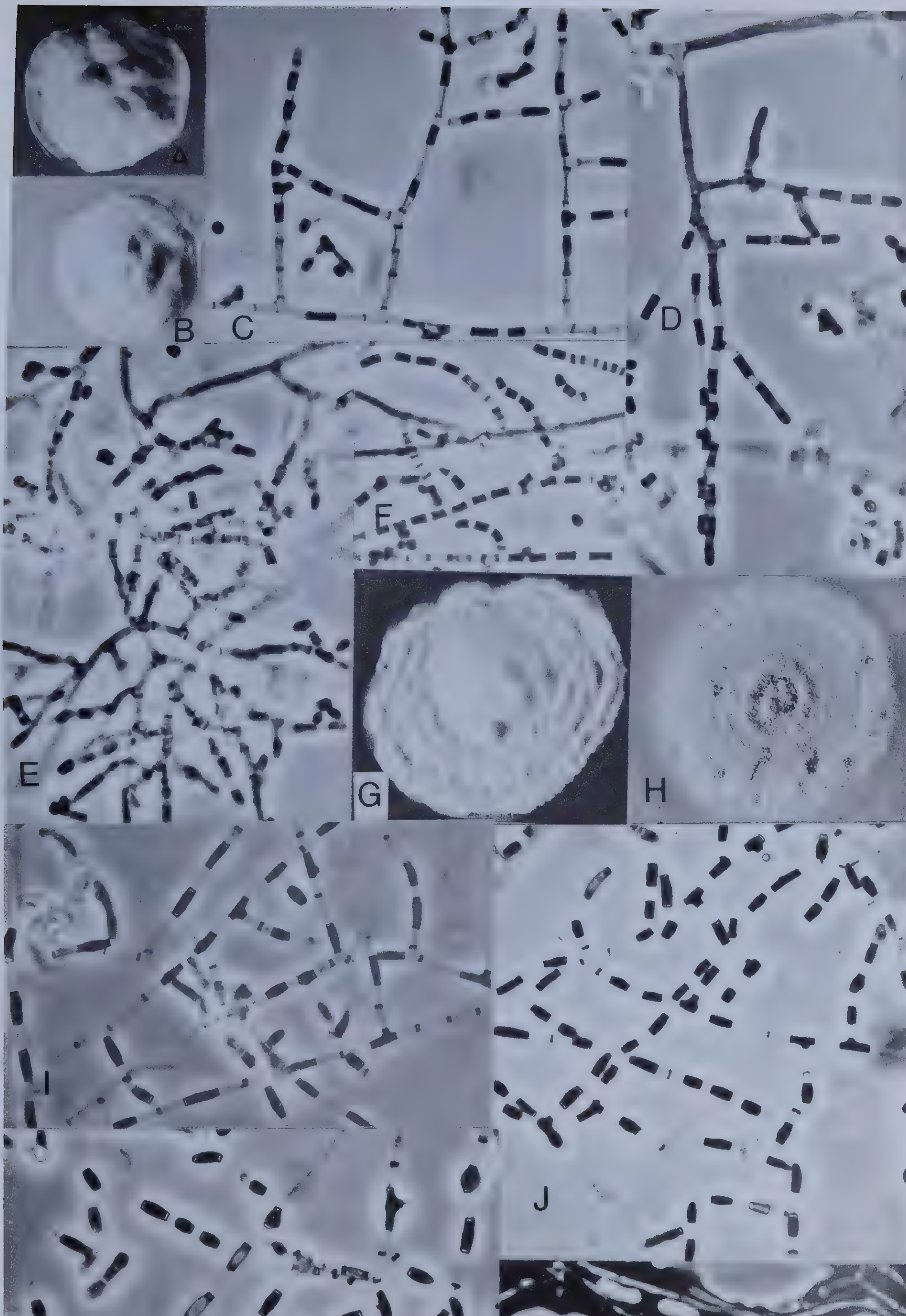
*M. flocciformis* digests cellophane after prolonged growth and is slightly keratinolytic.

## Material Examined

UAMH 675, wooden lemon storage bin, California, by P.R. Harding, 1959, from DAOM as 64000 (CMI 96741); UAMH 3273, TYPE, briny soil, Chateau-Saline, Lorraine, France, from J. Nicot, Laboratoire de Guffbogaurie, Paris; UAMH

Figure 17. A-E. _Malbranchea flocciformis_ (A-E-3273; F-675). G-L. _Malbranchea fulva_ (G,H-2851; I-1708; J-2849; K,L-3729). Figs. 17A-17B. Colonial morphology after 21 days at 25 C. A on PYE, B on cereal. Figs. 17C-17D. Alternate arthroconidia borne on straight primary hyphae and lateral branches. Fig. 17E. Repeated branching of fertile hyphae. Fig. 17F. Slightly curved sporogenous branches. Figs. 17G-17H. Colonial morphology at 21 days at 25 C. G on PYE, H on cereal. Figs. 17I-17J. Intercalary arthroconidia of straight, branched fertile hypha. Fig. 17K. Mature arthroconidia with refractile terminal walls. Fig. 17L. Orange brown slime surrounding vegetative hyphae. Colonies x 1.0, others x 830.

[illegible] *[illegible]lbranchea flocciformis* (A-E-3273; F-[illegible]). [illegible] *[illegible]chea fulva* (G,H-2851; I-1708; J-[illegible] [illegible] days [illegible] on PYE, B on cereal. Figs. 17C-[illegible] Alte[illegible]idia borne on straight primary hy[illegible] lat[illegible]l [illegible] Fig. 17E. Repeated branching [illegible] [illegible] slightly curved sporogenous br[illegible] [illegible] [illegible]straight, branched fertile hypha. [illegible] [illegible]nidia with refractile termi[illegible] [illegible] [illegible]thers x 830.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3785, salty soil, France, by J. Villoutreix, from CBS as 361.69

*Malbranchea fulva* Sigler et Carmichael sp. nov.

Description

Colonies on PYE (Fig. 17G) growing moderately quickly (38-44 mm in 21 days) are slightly raised with central tan or yellow umbo and four or five outer zones varying in color from tan or pale brown to yellow, and at the periphery white or hyaline, reverse dark tan or reddish brown. The zonate pattern is more distinct on half of the colony and the umbo may be situated slightly off center. The texture is powdery though the central umbo may be downy. A tan pigment diffuses slightly into the medium by 21 days.

On cereal agar (Fig. 17H) growth is as rapid (38-42 mm in 21 days) but flat with a small ridge surrounding a zone of less dense aerial growth at the center. The colony is tan or buff brown, reverse tan, powdery or granular. By 21 days, a slight amount of tan or brown pigment appears below the colony.

The maximum temperature for growth is close to 37 C, with some strains growing slightly (2-8 mm in 21 days) and others not at all. The optimum range is 25-30 C.

Hyphae bearing arthroconidia are straight, branched

(Figs. 17I,17J) septate 2-4um wide. Racket hyphae appear during early growth swelling to 5-6um in diameter. Septae formed in the fertile hyphae are thick (Figs. 17I,17J) suggesting double septae, and at maturity arthroconidia develop refractile terminal end walls (Fig. 17K). Arthroconidia are cylindrical or barrel shaped, hyaline, pale yellow, in mass yellow, smooth, 2-3(3.5)um x 3.5-8(11)um, sometimes remaining connected in 2's. Orange brown slime, occurring infrequently, surrounds some hyphae (Fig. 17L).

No other spore state was observed and an attempt to cross the isolates was unsuccessful.

Holotype: air sample, Dugway, Utah, by G.F. Orr, 1967, UAMH 2851 (DPG-167, 0-3042, NRRL 5160)

Discussion

*M. fulva* differs from the *M.* state of *Uncinocarpus reesii* in its slower growing tan colonies and restricted growth at 37 C. *M. fulva* lacks the arcuate fertile hyphae of *M. arcuata*.

Habitat and Activities

This species has been recovered from air (Orr and Kuehn, 1972), soil and dung, California, Utah and Colorado.

*M. fulva* is strongly keratinolytic, producing marked

digestion of hairs with some penetration of the hair by single hyphae. Cellophane is not attacked.

Material Examined

From soil: UAMH 1050, Riverside Co., California, by G.F. Orr, as O-759; UAMH 1708, Palo Verde, California, by G.F. Orr, 1957, as O-630; From air sample: UAMH 2849, wind tunnel contaminant, Utah, by G.F. Orr, 1967, as 4TL3; UAMH 2851, TYPE, Dugway, Utah, by G.F. Orr, 1967, as DPG-167 (O-3042, NRRL 5160); From dung: UAMH 3889, mouse, Fruita, Colorado, from Orr as O-1508; UAMH 3901, lizard, Palo Verde, California, from Orr as O-1319; From other sources: UAMH 3729, unknown, from Orr as DPG-45.

*Malbranchea gypsea* Sigler et Carmichael sp. nov.

Description

Colonies on PYE vary somewhat in growth rate, reaching a diameter of 17-39 mm in 21 days, but mostly 27-30 mm. Colonies (Fig. 18A) are chalky white or creamy white, reverse buff, downy or velvety, slightly raised, dome shaped with the surface deeply folded or convoluted, sometimes with prominent central umbo. The colony (Fig. 18B) of two strains (UAMH 1842 and 1843) was smoother with a hairy or coarsely woolly matted texture and glabrous margin.

On cereal, colonies (Fig. 18C) are slow growing (15-42

mm in 21 days, but mostly 27-32 mm) and appear white, reverse white or tan, powdery, downy, or floccose, almost flat or domed with small central umbo and small irregular folds. Tan pigment appeared below the colony of a single strain (UAMH 1975) by 21 days.

Most strains failed to grow at 37 C except UAMH 1841 and 1843 which showed scant growth. One strain (UAMH 1734) grew poorly at 30 C. The optimum temperature is 25-30 C.

Arthroconidia (Figs. 18D,18F), borne in an intercalary or terminal position on the straight primary hyphae or on short or long lateral branches, are separated by one or more alternate empty cells, or rarely, formed immediately adjacent to each other (Fig. 18E). Arthroconidia are cylindrical or slightly barrel shaped with the diameter slightly broader than the diameter of the interconnecting hypha (Figs. 18E,18F), hyaline, smooth, 2-2.5um x (2.5) 3-6 (9) um.

No other spore state was observed. An attempt to cross three isolates (UAMH 1841, 1843 and 1975) with each other was unsuccessful.

Holotype: soil, Bakersfield, California, by G.F. Orr, 1964, UAMH 1975 (VJC 4, 0-2565)

<u>Discussion</u>

The white colored colonies and lack of arcuate fertile

Figure 18. A-F. *Malbranchea gypsea* (A,C-UAMH 1975; B-1843; D-F-1841). G-I. *Malbranchea* state of *Coccidioides immitis* (3624). Figs. 18A-18C. Colonial morphology after 21 days at 25 C. A and B on PYE, C on cereal. Figs. 18D-18F. Arthroconidia borne on primary hyphae and straight lateral branches. Figs. 18G-18I. Closely spaced, barrel shaped arthroconidia borne on straight lateral branches. Colonies x 1.0, others x 830.

Figure [illegible] [illegible]. *Malbranchea gypsea* (A, [illegible]

Figure [illegible]. *Malbranchea* state of *Coccidioides* [illegible]

[illegible]

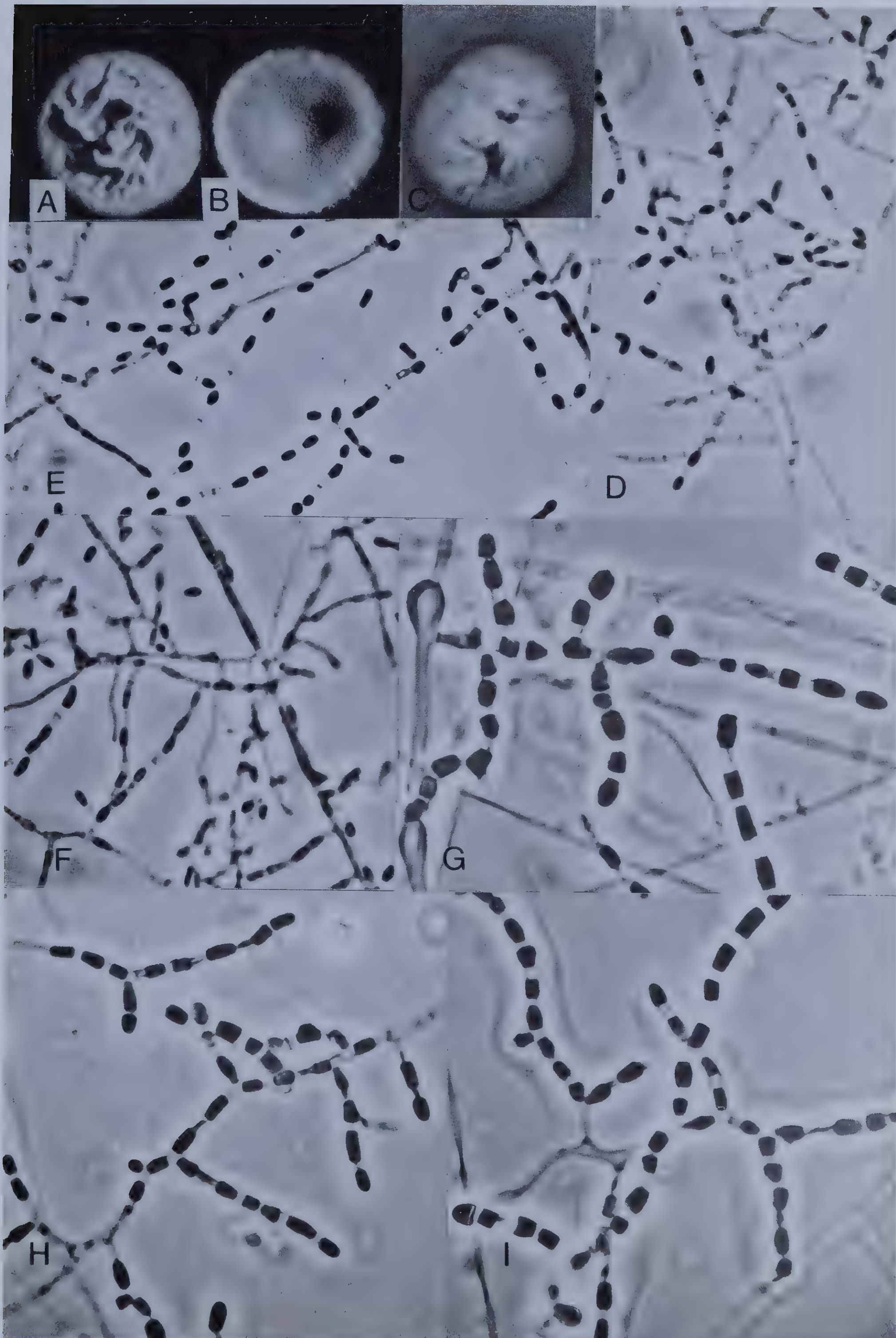
[illegible] B on PYE, C on cereal. Figs. [illegible]

[illegible] borne on primary hyphae and straight lateral

[illegible]

[illegible] borne on straight lateral branches. [illegible]

[illegible] x 830.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hyphae distinguish *M. gypsea* from most other species of *Malbranchea*. During early development, *M. gypsea* may be confused with the *Malbranchea* state of *Uncinocarpus reesii*, especially if the characteristic uncinate appendages of the latter are lacking. However, the *M.* state of *U. reesii* differs in forming arthroconidia which are slightly broader, 2.5-3um in diameter compared to 2-2.5um for *M. gypsea*; in growing more vigorously at both 25 C and 37 C; and in being markedly keratinolytic in contrast to *M. gypsea* which is cellulolytic.

## Habitat and Activities

Isolated from soil and cat hair, and recovered from lesions on the spleen and liver after passage through experimental animals, Utah and California (Orr, 1972).

*M. gypsea* is cellulolytic, weakening the cellophane membrane after prolonged growth. Keratin is not attacked.

## Material Examined

Isolates from soil passaged through experimental animals: UAMH 1841, California, from Orr as SFVH 129 (O-3226); UAMH 1842, California, from Orr as SFVH 130 (O-3283); UAMH 1843, California, from Orr as SFVH 149 (?O-1088); From other substrates: UAMH 1734, cat hair, by C.W. Emmons, from Orr as 'cat hair PBSF'; UAMH 1975, TYPE, soil, Bakersfield, California, by G.F. Orr, 1964 as VJC 4 (O-2565)

*Malbranchea* state of *Coccidioides immitis* Stiles in Rixford and Gilchrist 1896, Johns Hopkins Hospital Report 1:209-269

*Description*

The cultural morphology and pathology of *C. immitis* are described in medical mycology textbooks (see Rippon, 1974). Since central Alberta is not an endemic zone, the following description is based on two locally isolated strains (UAMH 3624 and 3667) from infected travellers. Slide cultures of these strains were kindly supplied by Dr. A. S. Sekhon (Provincial Laboratory of Public Health, Edmonton).

The arthroconidia are borne in an intercalary or terminal position on short or long, straight, lateral branches, arising at right angles from the vegetative hypha (Fig. 18G). The vegetative hyphae are narrower than the fertile branches, septate, hyaline, 2-4.5um in diameter, often with enlargements at the septae to 9um in diameter, and do not divide into arthroconidia. Arthroconidia (Figs. 18H,18I) are closely spaced, separated by short segments, hyaline, barrel shaped, cuneiform if terminal, 3-5(6)um x 3-8um, mostly 3.5-4.5um x 3-8um. The diameter of the arthroconidia is the same as the width of the fertile hypha, or only slightly larger.

Discussion

In its arthroconidium formation, C. immitis is close to the borderline between Malbranchea (conidia mostly less than 4um in diameter) and Sporendonema (conidia mostly more than 4um in diameter). However, the general appearance of the fertile hyphae and arthroconidia most closely resembles the other species of Malbranchea. Indeed, Huppert et al. (1967) apparently confused some isolates of other Malbranchea species with C. immitis and described them as variants of C. immitis. They reported that these unusual isolates produced coccidioidomycosis in mice, but made no mention of controls to rule out the possibility of accidental infection of their mice with C. immitis. Their figure 6 displays the distinctive arthroconidial morphology of Malbranchea dendritica. The type strain of M. dendritica (UAMH 2731) studied by Orr (1972) survived for long periods in mice and could be recovered from the spleen, liver and lung. However, no endosporulating spherules or other fungal elements were found in any tissue.

In the same study, Orr (1972) included three other arthroaleuriospore forming isolates (UAMH 1841, SFVH 129; UAMH 1842, SFVH 130; UAMH 1843, SFVH 149), none of which produced coccidioidomycosis in mice. These three strains, which have been included in the species Malbranchea gypsea, are not so distinctive in their microscopic morphology. However, Figure 7 of Huppert et al. (1967) suggests M.

gypsea and may be a photomicrograph of one of the above strains having SFVH numbers.

Three isolates were received from the CDC as C. immitis from Russia (UAMH 3595, 40-73-48553; UAMH 3596, 40-73-48555; UAMH 3602, 40-73-48554). They form chains of budding conidia and have been assigned to the form-genus Fusidium (Lechevalier, Lechevalier, Handley, Ghosh and Carmichael, 1975).

The arthroconidia of the Malbranchea state of C. immitis are broader than all other species of Malbranchea which are mostly less than 4um in diameter. Arthroconidia of M. sulfurea measure 2.5-4um but this species is thermophilic and forms arcuate fertile hyphae. The arthroconidia of the M. state of U. reesii are slightly narrower, 2.5-3.5um occasionally 4um in diameter and are separated by intervals of irregular length. In addition, the M. state of U. reesii regularly forms uncinate appendages in culture.

Malbranchea state of Uncinocarpus reesii

Sigler et Orr sp. nov.

Perfect State:

Uncinocarpus Sigler et Orr gen. nov.

Diagnosis

Ascocarps more or less spherical, reddish brown. Ascomatal initials on short stalks, bulbous. Peridial hyphae, smooth, aseptate, consisting of elongate appendages loosely intertwined. Free ends extending beyond the core of the ascocarp, uncinate, sometimes spiral. Asci subglobose, evanescent, 8-spored. Ascospores oblate, smooth, yellow to reddish brown. Malbranchea conidial state.

Type species: Uncinocarpus reesii Sigler et Orr sp. nov.

History

This fungus has been frequently isolated during surveys of soil and keratinous substrates. The imperfect state, most often encountered, has been ascribed to Chrysosporium, Malbranchea or various imperfect forms of Gymnoascaceae. The perfect state has been reported by various workers as a probable new genus of the Gymnoascaceae, but not yet described (see below).

Considerable attention has focused on the imperfect state because of its resemblance to the pathogenic Coccidioides immitis. Emmons (1954) studied several Gymnoascaceae isolated from organs of rodents and other animals. Some of these, which he called Myxotrichum formed the perfect state, whereas others, seemingly degenerate, produced only peridial appendages. This loss of fertility Emmons noted, was related to a change in colony color from

orange to white.

Examining two of Emmons' ascosporic isolates, Kuehn (1955a) described _Myxotrichum emmonsii_, later reduced to synonymy with _Auxarthron umbrinum_ (Boudier) by Orr and Plunkett (in Orr _et al_., 1963a). Both Kuehn, and Orr and Plunkett described an arthroconidial state, though neither reported the persistence of peridial appendages in degenerate strains.

However, Kuehn _et al_. (1964) discovered several occurrences of appendages in isolates similar to _Auxarthron brunneum_. In a later report, Emmons (1967) confirmed that his isolates earlier designated _Myxotrichum_ were _M. emmonsii_ Kuehn and again reiterated his observations of peridial appendages persisting in infertile strains. Emmons discerned little difference between _Auxarthron umbrinum_ and _A. brunneum_ and noted arthroconidia of both resembled _Coccidioides immitis_. Furthermore, Emmons found no evidence to suggest that appendages persisted in any other Gymnoascaceae maintained in culture.

Rees (1967a), surveying keratinophilic fungi from Australia isolated the imperfect state of ? _Gymnoascus uncinatus_ from _Rattus rattus_. In a subsequent study Rees (1967b) listed Genus A and noted its similarity to the previously isolated ? _G. uncinatus_ which formed only arthroconidia and trichomes in culture. He concluded the relationship of Genus A and the earlier reported ? _G._

*uncinatus* to *Gymnoascus uncinatus* appeared doubtful. Two of Rees' isolates (F143, UAMH 2845 and F122, UAMH 3484) called Genus A, studied by us, presented the first indication that the perfect state of the arthrosporic fungus forming appendages was not in fact *Auxarthron* as suggested by Emmons.

De Vroey (1970) reported the perfect state as a new genus of Gymnoascaceae from soil in Sulawesi (Celebes Islands). Caretta and Piontelli (1975) discovered an unidentified Gymnoascaceae and its imperfect *Chrysosporium* stage from woods in Italy.

Hubalek (1974a) reported several isolates of the imperfect state as a probable new species of *Auxarthron* from birds in Yugoslavia and Czechoslovakia.

## Description

Colonies on PYE (Fig. 19A) growing moderately rapidly (51-64 mm in 21 days) are creamy white or buff, never orange, reverse dirty yellow, domed and convoluted, or flatter with outward radiating folds, sometimes umbonate, downy or powdery. If powdery, cracks often appear near the center and along the folds.

At 37 C, colonies grow slowly (6-24 mm at 21 days), adhering poorly to the cellophane becoming heaped up and convoluted, downy, waxy or glabrous, or growing more rapidly

(Fig. 19B) (28-46 mm at 21 days) slightly raised, with numerous folds, white or tan, downy. Dark brown exudate droplets appear on the surface and a yellow pigment diffuses into the medium. This pigment can be detected with difficulty in some strains at 25 C, but is mostly obscured by the yellow color of the PYE.

Colonies on cereal (Fig. 19C) growing moderately rapidly (39-53 mm in 21 days) are creamy white, buff or tan, never orange, with white periphery, reverse the same. The texture is powdery or ganular, rarely downy, dense or patchy appearing mottled, occasionally zonate, flat with small central umbo and numerous tiny cracks or fissures on the surface.

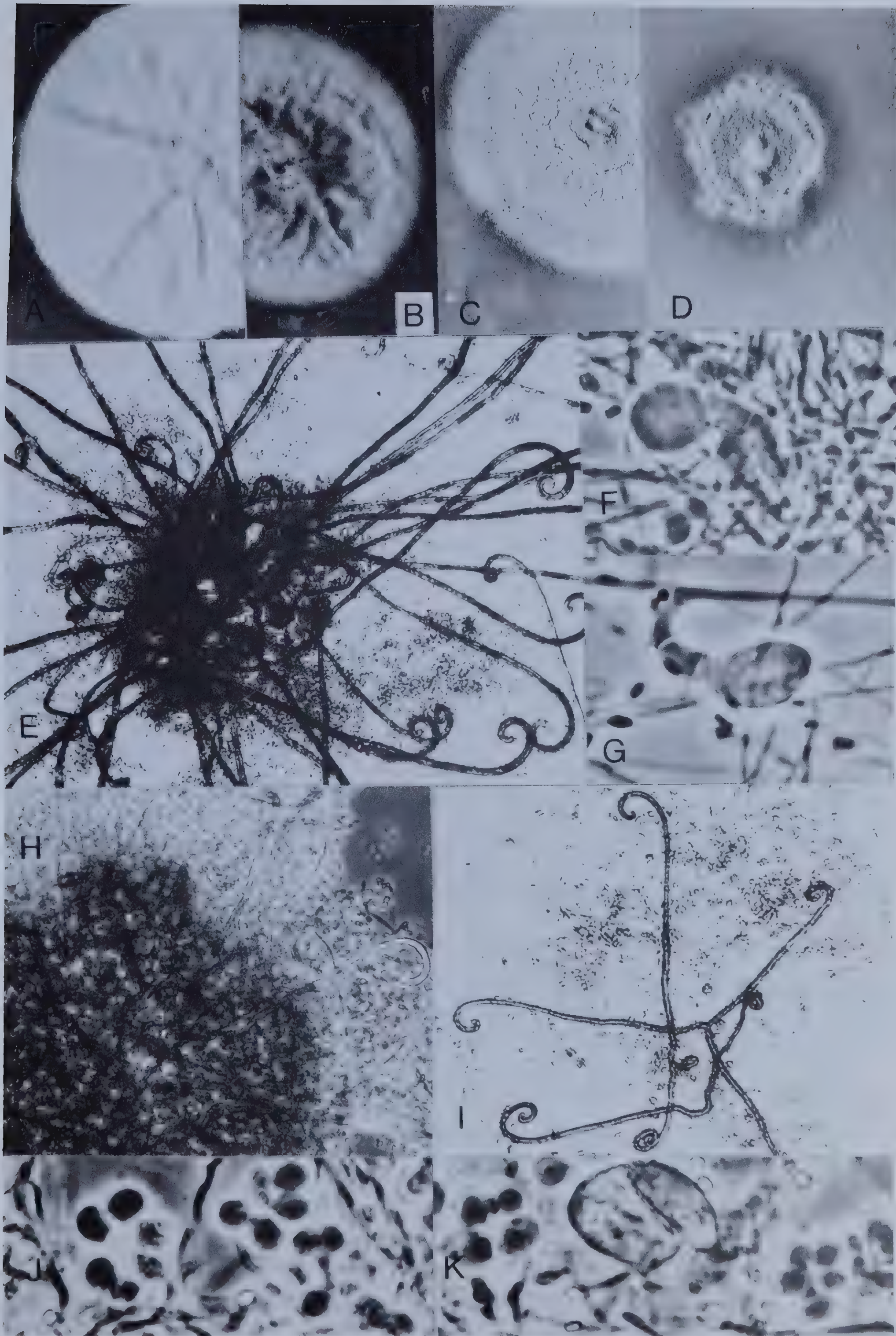
At 37 C, growth at 21 days is scant (5-10 mm) or restricted (14-26 mm). Colonies of the latter (Fig. 19D) are downy, floccose and white, or tan and granular, with a brown pigment diffusing into the medium below (Fig. 19D). This brown pigment is rarely observed at 25 C.

Heterothallic. Cleistothecia (Figs. 19E,19H) are discrete, reddish brown, globose, compacted in the center, 200-1000um, with appendages extending beyond 100-250um. Ascomatal initials, hyaline, arising as short branches, septate at first, with a bulbous enlarged tip, often completely inrolled (Figs. 19F,19G). Cleistothecium composed of a loose association of intertwined appendages, uncinate at each end, often forming one or more lateral

Figure 19. Uncinocarpus reesii (A-D-2847; E-2002x2847; F-3484; G-3573; H-2845; I-2855x3257; J,K-3881x2002). Figs. 19A-19D. Colonial morphology after 21 days, x 1.0. A and B on PYE, C and D on cereal. B and D at 37 C, others at 25 C. Fig. 19E. Fertile cleistothecium composed of loose association of uncinate appendages. x 210 BF. Figs. 19F-19G. Ascomatal initials, x 830. Fig. 19H. Cleistothecium, x 210. Fig. 19I. Intertwined branched uncinate appendages from a fertile cleistothecium, x 210 BF. Fig. 19J. Asci, x 830. Fig. 19K. Asci and ascomatal initial, x 830.

[illegible]

[illegible] cleistothecium [illegible] appendages. [illegible] x 830. [illegible] Fig. 191. [illegible] x 830. Fig. [illegible]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uncinate branches (Fig. 19I), smooth, thick walled, aseptate, reddish brown in color. The appendages are mostly uncinate, but occasionally spiral, wide at the tip, 5-8um, narrowing toward the center. No differentiated peridial hyphae are formed, though immature cleistothecia are often surrounded by a loose weft of hyaline hyphae bearing arthroconidia. Asci are evanescent, hyaline, arising on short stalks, subglobose, 7-9um in diameter (Figs. 19J,19K), 8-spored. Ascospores are yellow brown, reddish brown in mass, smooth, oblate, sometimes with a depression in one face (Figs. 20A,20B), 2.5-3um x 4-5um, mostly 2.5-2.8um x 4-4.5um.

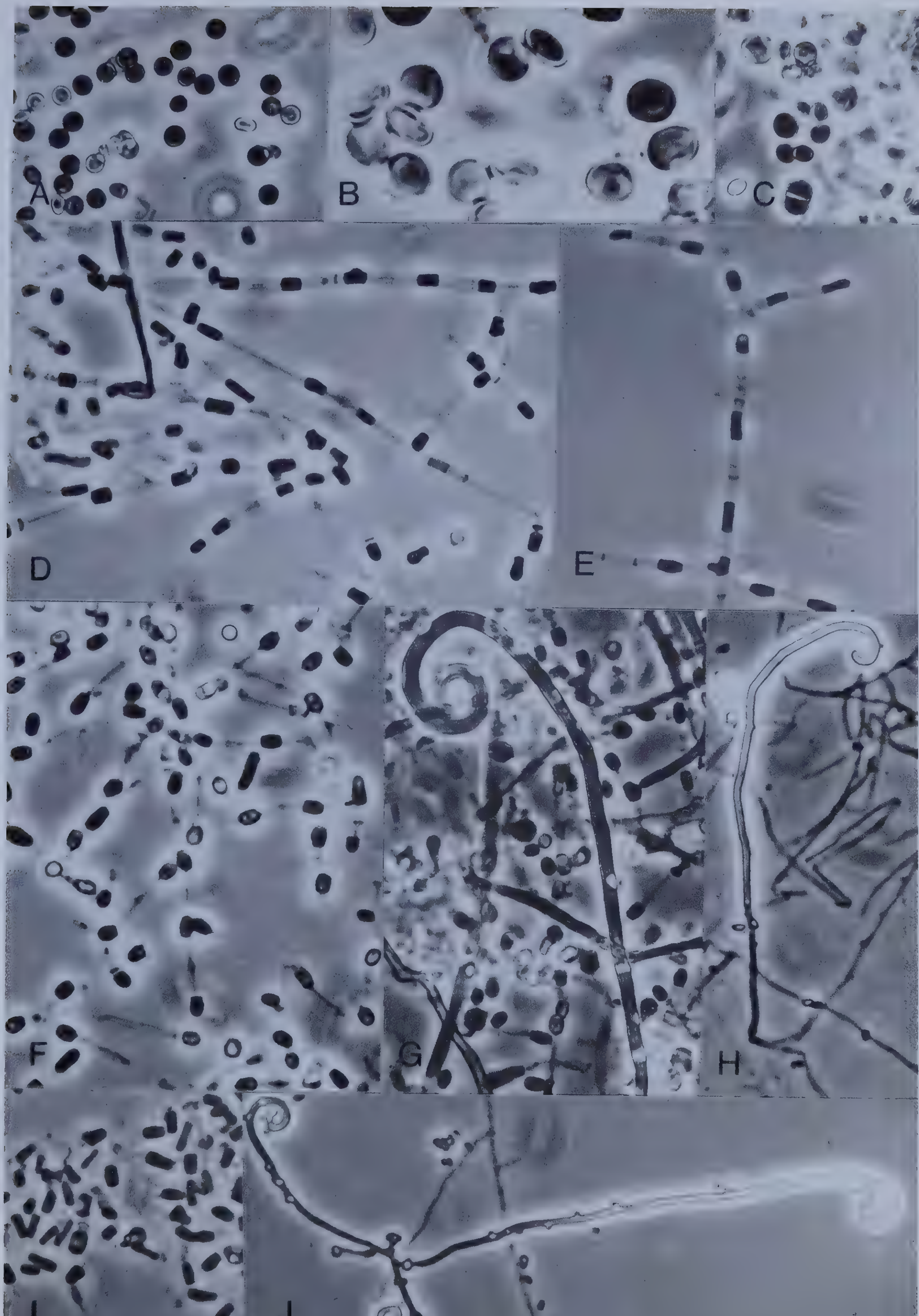
Arthroconidia (Figs. 20D,20E), borne in an intercalary position along the broader (2-5um) primary hyphae, or intercalarly or terminally on short or long lateral branches, are cylindrical, sometimes barrel shaped or cuneiform if terminal (Figs. 20F,20I). Separated by one or more segments of irregular length, arthroconidia are hyaline, later tan, smooth, 2-4um x 3-6(8)um, mostly 2.5-3.5um x 3.5-6um. Enlarged subglobose or broadly barrel shaped arthroconidia (Fig. 20C) are found in old cultures and in the vicinity of fertile cleistothecia. They are 5-8um in diameter, hyaline, mostly single celled. Racket hyphae may be present.

Uncinate appendages (Fig. 20G) are characteristic of the imperfect state, appearing in culture after 7-14 days.

Figure 20. *Uncinocarpus reesii* (A-C-1704x2050; D-2845; E-2991; F-3257; G-1704; H-3485; I-3703; J-2847). Figs. 20A-20B. Smooth oblate ascospores with a depression in one face. Fig. 20C. Ascospores and enlarged, 0 or 1 septate arthroconidia. Figs. 20D-20F. Arthroconidia borne on the primary hyphae or on straight lateral branches. Fig. 20G. Uncinate appendage of the *Malbranchea* state. Fig. 20H. Uncinate appendage arising as a thick walled extension from the vegetative hypha. Fig. 20I. Mature arthroconidia. Fig. 20J. Branched uncinate appendage of the *Malbranchea* state. B x 2300, H and J x 330, others x 830.

[illegible] ascospores with a [illegible]

[illegible] Ascospores and enlarged, [illegible] Figs. 20D–20F. [illegible] or on straight lateral [illegible] Fig. 20I. [illegible] uncinate appendage of the [illegible] and J x 330, others x 830.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The appendage, arising mostly as a thick walled, more dense extension from the primary hypha (Fig. 20H) or from a thick walled segment of the hypha resembling a foot cell, becomes uncinate at each end, often forming one or more lateral branches (Fig. 20J). The hypha bearing appendages continues to extend forming arthroconidia along its length. The appendages are mostly uncinate, occasionally spiral, aseptate, pale brown, in color, usually paler in color and slightly narrower in diameter at the tip (3-5um) than the appendages forming the cleistothecium.

Holotype: dried colonies of cross of single ascospore isolates UAMH 3880x3881, UAMH 3882

Mating types: UAMH 3880 '-', single ascospore isolate 'A' of UAMH 2845

UAMH 3881 '+', single ascospore isolate 'E' of UAMH 2845

## Discussion

Formation of cleistothecia in culture has been observed in two strains, UAMH 2845 and 3484. Single ascospore isolates of both were self-sterile, however (see results of mating tests).

The *Malbranchea* state of *Uncinocarpus reesii* is easily identified by its uncinate appendages. These appendages are lacking in all other *Malbranchea* species and have not been observed in any other conidial state of Gymnoascaceae

examined by us. Some strains lack appendages (UAMH 3257 and 3258), but nevertheless can be recognized by their arthroconidia and white or buff colonies.

*M. arcuata* differs in forming arcuate fertile hyphae. *M. fulva* grows more slowly at both 25 C and 37 C and its arthroconidia are slightly narrower and often refractile at the terminal ends. In addition, the arthroconidia of *M. fulva* are separated by relatively short segments, equivalent to or less than the length of an arthroconidium whereas the intervals between arthroconidia in *U. reesii* are longer and more irregular.

The genera of Gymnoascaceae are differentiated by the peridial hyphae, the shape and surface ornamentation of the ascospores and the ascomatal initials (Samson, 1972). On this basis, *Uncinocarpus* is most closely related to *Gymnoascus* Baranetzky, which also has oblate smooth ascospores. *Gymnoascus*, however, is differentiated by the net-like structure of its peridial hyphae which sometimes terminate in uncinate or elongate appendages, by its ascomatal initials which are coiled, and by its *Chrysosporium* conidial state (Orr *et al.*, 1963c; Samson, 1972). The peridial hyphae and reticulate or spiny, globose or oblate ascospores distinguish the genus *Auxarthron*. *Auxarthron* however, is related to *Uncinocarpus* in its keratinolytic activity, its formation of elongate uncinate appendages, and its *Malbranchea* conidial states.

*Petalosporus* (Ghosh, Orr and Kuehn, 1963) and *Shanorella* (Benjamin, 1956), two closely related genera, also form oblate ascospores, but differ from *Uncinocarpus* in their rudimentary peridial hyphae and coiled ascomatal initials. In addition, species of both genera are cellulolytic.

Furthermore, although heterothallic species are common in *Nannizzia*, *Arthroderma* and *Ctenomyces*, none have been reported so far in the other genera of Gymnoascaceae. However, there are several reports of loss of fertility in some species (Orr *et al*., 1963b; Apinis, 1964; von Arx, 1971; Samson, 1972). The *Myxotrichum* states of *Malbranchea flavoroseus* and *M. circinata* reported herein lacked ascospores, suggesting possible heterothallism.

## Results of Mating Tests

### A. Notes on techniques

The presence of fertile cleistothecia in two isolates of ? *Gymnoascus* species, associated with a *Malbranchea* conidial state, prompted initial investigation of compatibility among several similar *Malbranchea* strains. Fertile crosses confirmed the perfect state to be *Uncinocarpus reesii*.

Two methods of inoculation were first tested, the

premixed conidial suspension (method A) and the streak-plate method (method C). Method A, similar to that proposed by Padhye _et al_. (1973) had two disadvantages. If a large number of strains were to be tested, it was extremely time consuming, and the possiblity of error increased, either in labelling of the large number of tubes, or in transferring. However, the results of fertile crosses were similar in both methods (Table V) varying slightly in the number of cleistothecia formed. A single exception was the cross of UAMH 3703 x 2002 which failed to develop cleistothecia by method A.

Because of the disadvantages of method A subsequent studies utilized primarily method C and sometimes B, a more simple procedure for mixed suspensions (Table V).

The streak-plate method (C) overall had several advantages. Cleistothecia formed most commonly in the central region between the two strains. This easily found location with less dense vegetative growth increases the ease of observation. Cleistothecia were never formed in great abundance and when small were difficult to differentiate from clumps of conidia. Further, if strains were in fact inhibitory, a readily apparent demarcation zone could be observed between the tester strains. One of the problems associated with sexual crosses is the formation of profuse pleomorphic overgrowth which obscures cleistothecia. This degeneration occurred less rapidly in the streak-plate

cultures.

## B. Results of crosses

The compatibility among isolates of the *Malbranchea* state of *U. reesii* is summarized in Table VI. Of the 19 wild type isolates tested, six were found to be incompatible with all other strains and with each other. The incompatible strains, UAMH 160, 1273, 1706, 1955, 2992 and 3573, resembled the others in microscopic morphology although UAMH 3573 also formed ascomatal initials in abundance. All wild type isolates were self-sterile.

## C. F1 progeny

Single ascospore isolates of *Uncinocarpus reesii* required 30-32 hours at 30 C and up to 48 hours at 25 C before germination occurred.

Two single ascospore progeny of the self-fertile UAMH 2845, determined to be opposite mating types by back crossing with each other, were designated + (UAMH 3881) and - (UAMH 3880) mating types. All F1 progeny were self-sterile. These tester strains when back crossed with four wild conidial isolates known to be fertile, resulted in two fertile crosses, UAMH 2002 x 3881 and UAMH 2847 x 3880 (Table VI). UAMH 2854 and 3485 failed to cross with either mating type (Table VI).

TABLE V

COMPARISON OF ASCOCARP PRODUCTION IN UNCINOCARPUS REESII BY THREE METHODS OF INOCULATION

| Minus Strains | 2002 | | | 2050 | | | 3485 | | |
|---|---|---|---|---|---|---|---|---|---|
| Plus Strains | A | B | C | A | B | C | A | B | C |
| 1704 | | +++ | +++ | | | | | | |
| 2847 | +++ | | +++ | | +++ | +++ | +++ | | +++ |
| 2854 | +++ | | ++ | | | | + | | ++ |
| 2855 | | ++ | ++ | | | | | | |
| 2991 | ++ | + | | | | | | | |
| 3703 | - | ++ | | | | | | | |

Legend:

+ < 10 cleistothecia
++ 10-20 cleistothecia
+++ > 20 cleistothecia
- no fertile cleistothecia
blank: comparative tests not done

A Premixed suspension
B Suspensions mixed on plate
C Separate parallel streaks

TABLE VI
SUMMARY OF THE FERTILE CROSSES OF UNCINOCARPUS REESII

| | + Plus mating strains | | | | | | |
|---|---|---|---|---|---|---|---|
| - Minus Strains | 1704 | 2847 | 2854 | 2855 | 2991 | 3703 | 3881 |
| 2002 | +++ | +++ | +++ | ++ | ++ | +++ | +++ |
| 2050 | ++ | +++ | - | +++ | - | - | N |
| 2852 | + | - | - | +++ | - | - | N |
| 2862 | - | - | - | ++ | - | - | N |
| 3257 | - | - | - | +++ | - | - | N |
| 3258 | + | - | - | +++ | - | - | N |
| 3485 | + | +++ | ++ | + | - | - | - |
| 3880 | N | +++ | - | N | N | N | +++ |

+ < 10 cleistothecia
++ 10-20 cleistothecia
+++ > 20 cleistothecia
N Not tested

Six wild type isolates incompatible with all other conidial isolates failed to cross with either of these two tester strains. In addition, two F1 progeny from the cross of UAMH 2002 x 2847, found to be + (UAMH 3915) and - (UAMH 3916) mating types by back crossing with the parent strains, were also incompatible with all six wild type isolates.

Increased fertility of mating strains from the progeny of fertile crosses has been reported for other Gymnoascaceae (Kwon-Chung, 1971, 1972; Padhye and Carmichael, 1971, 1973). More work is required to determine if the failure of the six wild type isolates to cross is due to incompatibility or whether the *Malbranchea* state is a complex similar to that of *Microsporum gypseum* (Stockdale, 1963) or *Trichophyton terrestre* (Padhye and Carmichael, 1973).

## Pathogenicity

Though the pathogenicity of *U. reesii* has not been firmly established, it appears to be a transient inhabitant of man and animals, and can survive for extended periods in the tissue. Emmons (1954) recovered the organism from organs of rodents and other animals and from small omental abscesses at the site of intraperitoneal inoculation into mice.

Species of *Malbranchea* resembling *C. immitis* in appearance have been recovered from animal tissues, human sources and the spleen and liver of mice after

intraperitoneal inoculation (Kuehn *et al.*, 1964; Emmons, 1967; Orr, 1968, 1970). Orr (1972) reported that species of *Malbranchea* could be recovered from tissues of mice even when no gross lesions were apparent, and that fungal elements were not observed in any lesion.

## Habitat and Activities

*U. reesii* has been recorded from soil of desert and wooded areas, bat guano, keratinous substates such as feathers of wild birds and domestic fowl, organs of rodents and human sources. It appears to have a worldwide distribution.

This species attacks hair vigorously, digesting it with the aid of penetrating bodies. Cellophane is not attacked.

## Material Examined

Single ascospores isolates, Alberta, 1975: UAMH 3882, TYPE, dried colonies of cross of UAMH 3880 x 3881; UAMH 3880, - mating type, single ascospore isolate 'A' of UAMH 2845; UAMH 3881, + mating type, single ascospore isolate 'E' of UAMH 2845; UAMH 3883, - mating type, single ascospore isolate 'B' of UAMH 2845; UAMH 3884, - mating type, single ascospore isolate 'C' of UAMH 2845; UAMH 3915, + mating type, single ascospore isolate 'E' of cross of UAMH 2002 x 2847; UAMH 3916, - mating type, single ascospore isolate 'A' of cross of UAMH 2002 x 2847; From keratinous material (self-fertile

isolates): UAMH 2845, feathers, Australia, by Rees (F143), 1967, from Orr as 0-3406 (NRRL A-14871, QM 9335); UAMH 3484, feathers, Australia, by Rees (F122), from Orr as 0-3144 (NRRL A-14870); From keratinous material: UAMH 2862, feathers, Australia, by Rees (F122), ?1965, from Orr as F122; UAMH 3485, feathers, Australia, from Orr as 0-3047; UAMH 3843, feathers of *Corvus monedula*, Krivogastani, Yugoslavia, by Hubalek, 1968, as 193D (0-1296); UAMH 3846, nest of *Carduelis cannabina*, Valtice, Czechoslovakia, by Hubalek, 1970, as 539A (0-1304); 0-3367, feather of Jackdaw, Yugoslavia, by Hubalek, (766A), from Orr; From rodent lung: UAMH 160, *Sylvilagus auduboni*, Texas, by C.E. Emmons, 1949 as C 3716; From unknown sources: UAMH 2991, from R.S. Pore, W. Virginia Medical Center, Morgantown as 707; UAMH 2992, from R.S. Pore as 718; From human sources: 0-2559, sputum, Missouri, by Bransberg (118), from Orr; 0-3222, child's hair, Argentina by Negroni (274), from Orr; 0-3718, child's hair, Argentina by Negroni (276), from Orr; 0-782, child's hair, Argentina by Negroni(255), from Orr; From soil, California isolates: UAMH 1704, by Orr 1960, as 0-2080; UAMH 1706, by Orr, 1957, as 0-482a; 0-216, by Orr; 0-2569, by Orr; Hungarian isolates: UAMH 2847, fabric bait, by Orr, 1967 as 0-3513; UAMH 2854, fabric bait, by Orr, 1967, as SZ 284; UAMH 2855, fabric bait, by Orr, 1967 as SZ 303; Italian isolates: UAMH 2002, by Varsavsky, 1964, from Orr, EV 1-14; UAMH 2050, from Varsavsky as I-79; UAMH 2852, by Varsavsky, 1964, from Orr as I26; UAMH 3573, by Varsavsky, from Orr as

0-3407; UAMH 3819, by Varsavsky (I5), from Orr as 0-3344; UAMH 3820, by Varsavsky (I15), from Orr as 0-3233; UAMH 3918, Pavia, by Caretta (E 21 +), from Orr as 0-1322; 0-1323, Pavia, by Caretta (E 25), from Orr; 0-1324, Pavia, by Caretta, (E 9 I+), from Orr; South American isolates: UAMH 1955, Argentina, by Varsavsky, from Orr as EV-5U (0-3597); 0-3451, Argentina by Negroni (408), from Orr; UAMH 1273, Venezuela, by Pollak (5770) from L. Ajello, CDC as 45-36-62; 0-2558, Mexico, Plunkett (707), from Orr; Indian isolates: UAMH 3257, from H.C. Gugnani, National Institute of Communicable Diseases, Delhi, as S673; UAMH 3258, from Gugnani as S1038; 0-2081, by Garg (1045), from Orr.

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# APPENDIX

Figure 21. Colonies of _Malbanchea_ species and _Auxarthron conjugatum_ after 21 days at 25 C, except K at 37 C. Fig. 21A. _M_. _albolutea_ (UAMH 1846 on PYE). Fig. 21B. _M_. _arcuata_ (2983 on PYE). Fig. 21C. _M_. _arcuata_ (3877 on cereal). Fig. 21D. _M_. _circinata_ (3589 on cereal). Fig. 21E. _M_. _aurantiaca_ (3705 on PYE). Fig. 21F. _M_. _aurantiaca_ (3660 on cereal). Fig. 21G. _M_. _fulva_ (2849 on PYE). Fig. 21H. _M_. _chrysosporoidea_ (2288 on PYE). Fig. 21I. _M_. _chrysosporoidea_ (2288 on cereal). Fig. 21J. _Auxarthron conjugatum_ (3817 on PYE). Fig. 21K. _M_. _sulfurea_ (3747 on PYE). Fig. 21L. _M_. _flava_ (2865 on PYE). Fig. 21M. _M_. state of _Uncinocarpus reesii_ (2847 on PYE). Fig. 21N. _M_. _flavoroseus_ (1051 on cereal). Fig. 21O. _M_. _pulchella_ (1560 on cereal). Fig. 21P. _M_. _flocciformis_ (3273 on PYE). Fig. 21Q. _M_. _gypsea_ (1841 on cereal). All x 0.60.

[illegible] of [illegible] species and Au[illegible] [illegible] days at 25 C, except K at [illegible] [illegible]. albolutea (UAMH 1846 on PYE). Fig. [illegible] (on PYE). Fig. 21C. M. [illegible] [illegible] Fig. 21D. M. circinata (3589 on [illegible] [illegible]. aurantiaca (3705 on PYE). Fig. [illegible] (on cereal). Fig. 21G. [illegible] [illegible] Fig. 21H. M. chrysosporoidea ([illegible] [illegible] [illegible] conjugatum (3817 on PYE). Fig. [illegible] [illegible] on PYE). Fig. 21L. M. flava (2865 [illegible] [illegible] state of [illegible]carpus [illegible] [illegible] [illegible] (1560 on cereal). Fig. 21[illegible] [illegible] (3373 on PYE). Fig. 21Q. M. [illegible] [illegible] X 0.60.


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INDEX OF GENERA AND SPECIES

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